Domestic dogs in wolf grounds: insights on niche overlap between dogs and wolves in human dominated landscapes.

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Todas as correções determinadas

pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

MSc

“Every individual matters.

Every individual has a role to play.

Every individual makes a difference.”

Jane Goodall

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Abstract

Mammalian large carnivores, such as wolves, have a relevant role in ecosystems by contributing to maintaining their structure, operation, and biodiversity. Interspecific interactions are crucial in ecosystem functioning, with competition being one of the main interactions between wolves and mesocarnivores. In human-dominated landscapes, the most important mesocarnivore involved in relationships with wolves is the domestic dog. However, the literature regarding the ecological interactions between wolves and free-ranging dogs is scarce. The present work address this topic by focusing three wolf packs occurring in sympatry with domestic dogs in the region at south Douro river, in order to evaluate the overlap in their spatial, temporal and trophic niches.

In particular, the main goals of this study were: i) estimate the abundance of wolves and dogs, based on genetic individual identifications of scats collected between 2014 and 2018 (n=263 for wolves and 343 for dog), questionnaires to local institutions (n=10 surveyed municipalities) and genetic identification from collected saliva from dogs (n=15 sampled dogs); ii) evaluate space use and habitat selection by both canids, based on scats with individual genetic identification and using Spatial Capture-Recapture methods; iii) determine their circadian activity patterns through camera trapping data on wolf breeding areas (n=26 for wolves and 125 for dog); and iv) assess diet composition in wolves and dogs, based on the analysis of scats genetically validated collected between 2014 and 2018 (n=247 for wolves and 270 for dog).

Results showed that the estimated population size of wolves in the study area has stable numbers, with more males (12 individuals) than females (8 individuals). On the other hand, dogs have much higher and more irregular values, with males varying between 48 to 216 individuals and females between 38 to 132 individuals. Dogs have a much higher density (25 times higher than wolf density), and are much more dispersed in the study area than wolves. There is a relation between a higher wolf density at higher altitudes and closer to human build up, while dogs have a higher predicted density at longer distances from human build up and no relation with distance to wolf breeding sites. The relationship between the spatial predictions of wolves and domestic dogs is inversely proportional, as our results predicted that the density of dogs decreases along with an increase in wolf density. The spatial interaction between the two species found that dogs are widely present and dispersed inside pack´s territories, although a spatial segregation of dogs was observed at wolf breeding sites, suggesting a higher plasticity and dominant character in wolves.

The circadian activity revealed that wolves show mainly a nocturnal activity pattern while domestic dogs shown a clear diurnal activity. There were significant differences in the activity patterns between the two species, with dogs having a higher number of active hours per day than wolves, and with both canids being only 34% active simultaneously during a circadian cycle. These results suggest that wolves seem to adjust their activity patterns in order to temporally segregate from humans, while dogs adjust their activity pattern in relation to their dependency to humans for food and shelter.

The global results on diet composition showed a high similarity in the feeding habits of both canids in the study area (Pianka´s index = 0.813), although some dietary differences occur between wolf packs, sampling years and seasons. The main dietary differences are a higher consumption of domestic ungulates by dogs (78.5% of consumed Biomass) than wolves (58.5% of consumed Biomass), while dogs have lower consumption of Lagomorphs (5.3% of consumed Biomass) compared to wolves (20.7%. of consumed Biomass). Results also showed that wolves kill and consume domestic dogs. These findings suggest that both canids feed on the same resources, either by a predatory or scavenging behaviour.

Overall, we found a niche overlap between sympatric domestic dogs and wolves at the trophic and spatial levels but not at the temporal level, corroborating the different hypotheses related to their ecological interactions. This study provided valuable insights on the resource competition and space-temporal interactions between these two close-related canids. Furthermore, this study provides support for the need of several management actions, either in the study area or at a wider context, in order to assure the long-term survival of wolves in human-dominated landscapes.

Key words: Iberian wolf, domestic dog, competition, population estimates, habitat selection, circadian activity, diet, niche overlap.

Resumo

Os grandes mamíferos carnívoros, como os lobos, têm um papel relevante nos ecossistemas, contribuindo para a manutenção da sua estrutura, operação e biodiversidade. As interações interespecíficas são cruciais no funcionamento do ecossistema, sendo a competição uma das principais interações entre lobos e mesocarnívoros. Em paisagens dominadas por humanos, o mesocarnívoro mais importante envolvido em relacionamentos com lobos é o cão doméstico. No entanto, a literatura sobre as interações ecológicas entre lobos e cães em liberdade é escassa. O presente trabalho aborda este tema focalizado em três alcateias que ocorrem em simpatria com cães domésticos na região sul do rio Douro, a fim de avaliar a sobreposição dos seus nichos espaciais, temporais e tróficos.

Em particular, os principais objetivos deste estudo foram: i) estimar a abundância de lobos e cães, com base em identificações genéticas individuais de dejetos recolhidos entre 2014 e 2018 (n = 263 para lobos e 343 para cães), questionários para instituições locais (n = 10 municípios) e identificação genética da saliva coletada de cães (n = 15 cães amostrados); ii) avaliar o uso do espaço e a seleção de habitat por ambos os canídeos, com base em dejetos com identificação genética individual e utilizando métodos de captura-recaptura espacial; iii) determinar padrões de atividade circadiana das duas espécies através de dados de armadilhagem fotográfica em áreas de reprodução de lobos (n = 26 para lobos e 125 para cães); e iv) avaliar a composição da dieta em lobos e cães, com base na análise de dejetos geneticamente validados coletados entre 2014 e 2018 (n = 247 para lobos e 270 para cães).

Os resultados mostraram que o tamanho estimado da população de lobos na área de estudo apresenta números estáveis, com mais machos (12 indivíduos) do que fêmeas (8 indivíduos). Por outro lado, os cães apresentam valores muito mais elevados e mais irregulares, com os machos a variar entre 48 a 216 indivíduos e as fêmeas entre 38 a 132 indivíduos. Os cães têm uma densidade muito maior (25 vezes maior do que a densidade do lobo) e estão muito mais dispersos pela área de estudo do que os lobos. Há uma relação entre uma maior densidade do lobo em altitudes mais altas e mais perto de construções humanas, enquanto o cão apresenta uma maior densidade prevista em distâncias mais afastadas de construções humana, não havendo relação com a distância para os locais de reprodução do lobo. A relação entre as previsões espaciais de lobos e cães domésticos é inversamente proporcional, pois os nossos resultados previram que a densidade dos cães diminui a par de um aumento da densidade dos lobos. A interação espacial entre as duas espécies revelou que os cães estão amplamente presentes e dispersos dentro dos territórios das alcateias, embora uma segregação espacial dos cães tenha sido observada nos locais de reprodução dos lobos, sugerindo uma maior plasticidade e caráter dominante nos lobos.

A atividade circadiana revelou que os lobos apresentam principalmente um padrão de atividade noturna, enquanto os cães domésticos apresentam uma clara atividade diurna. Houve diferenças significativas nos padrões de atividade entre as duas espécies, com os cães tendo um número maior de horas ativas por dia do que os lobos, e só em apenas 34% do tempo é que ambos os canídeos estão ativos simultaneamente. Estes resultados sugerem que os lobos parecem ajustar os seus padrões de atividade a fim de se segregarem temporalmente dos humanos, enquanto os cães ajustam o seu padrão de atividade aos humanos de forma a dependerem dos mesmos para alimentação e abrigo.

Os resultados globais sobre a composição da dieta mostraram grande similaridade nos hábitos alimentares dos dois canídeos da área de estudo (índice de Pianka = 0,813), embora ocorram algumas diferenças na dieta entre as alcateias, anos de amostragem e estações. As principais diferenças na dieta são um maior consumo de ungulados domésticos por cães (78,5% da biomassa consumida) do que lobos (58,5% da biomassa consumida), enquanto que os cães têm menor consumo de lagomorfos (5,3% da biomassa consumida) em comparação com os lobos (20,7% .de biomassa consumida). Os resultados também mostraram que os lobos matam e consomem cães domésticos. Estas descobertas sugerem que ambos os canídeos se alimentam dos mesmos recursos, seja por um comportamento predatório ou de necrofagia.

No geral, encontramos uma sobreposição de nicho entre cães domésticos simpátricos e lobos nos níveis trófico e espacial, mas não no nível temporal, corroborando as diferentes hipóteses relacionadas com as suas interações ecológicas. Este estudo forneceu informações valiosas sobre a competição por recursos e as interações espaciotemporais entre estes dois canídeos. Além disso, este trabalho fornece suporte para a necessidade de diversas ações de gestão, seja aplicando-as à área de estudo ou noutro contexto mais amplo, a fim de garantir a sobrevivência a longo prazo dos lobos em paisagens de domínio humano.

Palavras-chave: lobo ibérico, cão doméstico, competição, estimativas populacionais, seleção de habitat, atividade circadiana, dieta, sobreposição de nicho.

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List of Abbreviations

ACHLI – Associação de Conservação do Habitat do Lobo Ibérico

Biomass – Consumed Biomass

CIBIO – Research Centre of Biodiversity and Genetic Resources, University of Porto

EDF – Encounter Data File

F.O. – Frequency of Occurrence

GIS – Geographic Information Systems

GPS – Global Positioning Systems

ICNF – Institute for Nature Conservation and Forest

N.O. – Niche overlap

SCR – Spatial Capture-Recapture

SIAC – Sistema de Informação de Animais de Companhia

TDF – Trap Deployment File

1. Introduction

1.1. The role of large carnivores in ecosystems

Large carnivores, usually defined as mammalian predators with more than 20 kg (Carbone *et al.,* 1999), have a relevant role in ecosystems, contributing to maintain their structure, function and associated biodiversity (Ritchie *et al.,* 2012). These predators are at the top of the food chain, often regulating prey populations and indirectly influencing competitors, such as mesocarnivores and vegetation cover, through top-down effects along trophic cascades (Terborgh *et al.,* 1999; Ray *et al.,* 2013). These top-down effects enforced by large carnivores on species at lower trophic levels can be imposed directly and indirectly (Terborgh *et al.,*1999). Through predation, carnivores may directly reduce the number of prey and modify their population structure (e.g. by killing older or young animals). Simultaneously, large carnivores may directly influence prey and competitors, as they induce changes in their behaviour by altering, for example, habitat selection (Laundre *et al.,* 2010) or diel activity patterns (Monterroso *et al.,* 2013), thus becoming less vulnerable to predation (del Rio *et al.,* 2001). In turn, large carnivores can be regulated by bottom-up effects, induced by lower trophic levels or factors influencing the dynamics of species interactions along trophic webs, such as habitat productivity or emergent diseases (Elmhagen & Rushton, 2007; Monterroso *et al.,* 2016a).

Predators are divided in two main groups based on their different trophic roles on food webs: top predators and mesopredators (Roemer *et al.,* 2009; Newsome *et al.,* 2017). Top predators are positioned in the superior position in the trophic web, occurring at lower densities and normally selecting larger prey (Ritchie & Johnson, 2009). Mesopredators are usually mid-ranking carnivores with a more generalist behaviour (Roemer *et al.,* 2009) and often suppressed by the largest or competitively dominant top predators (Levi & Wilmers, 2012; Newsome *et al.,* 2017).

1.2. Interspecific interactions between carnivores

One of the main interspecific interactions among mammalian predators is competition and happens when individuals from different species (interspecific competition) contest for a limited resource (Birch, 1957). Competition can be classified into: i) interference competition, when individuals directly affect the other competitors, such as aggression between them (Berger & Gese 2007); ii) exploitative competition, when individuals affect indirectly the other competitors by reducing the availability of a shared resource (Tilman, 2004); and iii) apparent competition, when negative indirect interactions between victim species arise due to other species that they share as natural enemies (Holt, 1977; Bonsall & Hassel, 1997). An extreme phenomenon of interspecific competition for a limited resource happens when species kill and often consume other from the same trophic level, called interspecific killing (Polis *et al.,* 1989; Lourenço *et al.,* 2013). The fear of interspecific killing results in avoidance behaviours (Polis & Holt, 1992; Newsome *et al.,* 2017), which may strongly affect species occurrence and habitat selection. This phenomenon is common among predators and may be responsible for more than 60% of known mortality in some carnivore species (Palomares & Caro, 1999). In fact, canids are one of the carnivore families most involved in this type of lethal interactions as they are represented by several mesocarnivores and top predators (Palomares & Caro, 1999; Mohammadi *et al.,* 2017).

However, there are other interactions between carnivores besides competition. High abundance of mesopredators can also influence populations of top predators, particularly due to hybridization between closed-related species, as frequently reported for canids (Bohling & Waits, 2011) or by being reservoirs for several pathogens (e.g., sarcoctic mange, rabies, parvoviruses and distemper virus) to which larger predators can be highly susceptible, given their lower abundance and reproductive rates (Roemer *et al.,* 2009; Krofel *et al.,* 2017). Furthermore, in areas where large carnivores become scarce or extirpated, populations of mesopredators tend to expand and reach high densities with several ecological and socio-economic impacts, a phenomenon known as mesopredator release (Prugh *et al*., 2009; Roemer *et al.,* 2009; Krofel *et al.*, 2017). These ecological interactions can become particularly relevant in human dominated landscapes, where populations of mesocarnivores are usually abundant, while large carnivores' populations are often reduced due to habitat loss and human persecution (Prugh *et al.,* 2009; Ripple *et al.,* 2014; Newsome *et al.,* 2017). Additionally, in landscapes with strong human activity, as is Europe, another mesocarnivore species is often abundant: the domestic dog (*Canis familiaris*). However, there is limited knowledge about its interactions with large carnivores, particularly with a closely related canid, the grey wolf (*Canis lupus*).

1.3. Wolves and domestic dogs in human-dominated landscapes

The grey wolf is one of the most widely distributed terrestrial mammals (Mech, 1970; Mech & Boitani, 2003). Despite being extirpated from most of its' historical range due to direct persecution and habitat destruction, wolves are now recovering and occupying most of their original distribution, including human-dominated landscapes (Mech & Boitani, 2003; Hunter, 2019). The grey wolf is a top predator with high adaptability, which allows him to adapt its circadian activity and spatial behaviour in order to occur under reasonable levels of human disturbance and in strongly humanized habitats, as long as food and refuge are available (Mech & Boitani 2003; Theuerkauf *et al.,* 2003a). Wolves feed mostly on wild ungulates (e.g. deer, moose or wild boar). Although, when these prey are scarce, the ecological plasticity of this carnivore allows it to take advange of a wide variety of other prey locally available, such as lagomorphs, rodents and livestock (Meriggi & Lovari, 1996; Zlatanova *et al.,* 2014; Newsome *et al.,* 2016). In fact, wolf predation on livestock constitutes the main driver for conflicts with humans' interests, which often motivate direct persecution towards this large carnivore (Mech & Boitani, 2003). Although livestock species, such as cattle, sheep and goats, are the main domestic animals consumed by wolves (Newsome *et al.,* 2016), there are also reported attacks and consumption of domestic dogs, which raises further adverse attitudes towards wolves (Fritts & Paul, 1989). In fact, wolf predation on domestic dogs have low public acceptance due to the strong emotional connection with these pets (Mech, 1995; Naughton-Treves *et al.,* 2003). Also, important economic losses to dog owners, particularly when it involves valuable dog breeds for hunting, transport or other human activities can occur (McNay, 2002; Backeryd, 2007).

Besides predation, domestic dogs can be involved in other types of interspecific interactions with wolves, such as competition and possible occurrence of interbreeding (Mech, 1970; Fritts & Paul, 1989). Domestic dogs are reported to compete with the Indian wolf for prey, such as offspring of blackbuck (*Antelope cervicapra*) (Jhala, 1993). Although there is poor knowledge on this type of wolf-dog interactions from other regions, it is expected to be frequent given the results from studies focusing other wolf-like canids. As an example, domestic dogs compete with the Ethiopian wolf (*Canis simensis*) for rodents (Sillero-Zubiri, 1994) and are reported to persecute Ethiopian wolves when these wander around human settlements, suggesting that domestic dogs can displace the Ethiopian wolf from their natural habitat (Atickem *et al.,* 2010).

On the contrary, hybridization between wolves and dogs has been widely studied and seems to be a frequent event, as these two taxa are closely related and produce fertile descendants (Lehman *et al.,* 1991; Wayne *et al.,* 1997). As an example, in Italy the number of free-ranging dogs is estimated to surpass the number of wolves by a factor of more than 100, showing that the combination of high dog densities with the low-density and fragmented wolf populations, intensify the risk of hybridization with dogs leading to negative consequences (Randi & Lucchini 2002). Furthermore, the difficult recognition of hybrids from parental species, may result in much higher occurrence of hybrid wolves in Europe than reported so far (Andersone *et al.,* 2002; Randi & Lucchini 2002; Vilà *et al.,* 2003). Therefore, hybridization between dogs and wolves raise several conservation and management issues as may lead to loss of genetic integrity in endangered wolf populations, and hybrid individuals can be more prone to occur near human settlements, promoting conflicts that may end to affect pure wolves (Allendorf *et al.,* 2001; Donfrancesco *et al.,* 2019).

Boitani (1983) conducted one of the first studies on the relationship between wolves and domestic dogs, with the purpose of obtaining basic data on dog numbers and biology, by resorting to questionnaires, radiotelemetry, and field census. The results showed that the feeding ecology of dogs and wolves was very similar, raising long-term conservation issues for the wolf due to competition and interbreeding with free-ranging dogs (Boitani, 1992). In Israel, Mendelssohn (1983) also suggests that the competition between dogs and wolves is one of the causes for the decreasing numbers of the remaining wolf populations. A study conducted in Poland, showed that free-ranging dogs are widespread and abundant, often killing wildlife and livestock thus competing with wolves (Wierzbowska *et al.,* 2016). Additionally, this study reported that dog predation, combined with human's harvest, may cause unsustainable off-take rates in some important game species, and reinforces the lack of studies regarding the ecological impact of domestic dogs (Wierzbowska *et al.,* 2016). Furthermore, the similar killing patterns between dogs and wolves may result in a significant proportion of ungulate predation by dogs being misleadingly attributed to the wolf (Echegaray & Vilà, 2010).

1.4. Ecological impacts of domestic dogs

Domestic dogs have a worldwide distribution and are often confined to the proximity with humans, who afford them food and protection (Vanak & Gompper, 2009). Domestic dogs can be categorized as pet, stray, or feral dogs, mainly distinguished by their dependence on humans, in a descending order correspondingly (Green & Gipson, 1994). Domestic dogs become stray when are pets that have strayed from or are abandoned by their owners, or feral, when live and breed without human contact or direct interference (Miklósi, 2015). Both stray and feral dogs continue indirectly dependent upon humans for food (Boitani & Ciucci, 1995), but as they roam freely can heavily predate on both wildlife and livestock (Denney, 1974), as well as influence prey activity and habitat use (del Rio *et al.,* 2001; Lenth *et al.,* 2008). Pet dogs despite being kept as a human companion, can also have functions that can implicate a stronger interaction with wildlife, such as hunting or livestock guarding dogs (Lescureux & Linnell, 2014).

Free-ranging dogs are known for attaining a high predation pressure on wildlife, particularly when occurring at high densities, becoming efficient predators of wild ungulates, such as red deer (Vanak & Gompper, 2009; Duarte *et al.,* 2016). Also, in Brazil, domestic dogs are reported to have a strong predatory impact on local wildlife, including inside remote protected areas (Lacerda *et al.,* 2009). This study also showed that the occurrence of the maned wolf (*Chrysocyon brachyurus*) was negatively associated with the presence of dogs, as these wild canids seemed to avoid areas used by dogs, particularly near garbage dumps (Lacerda *et al.,* 2009). Additionally, Butler and du Toit, (2002) reported necrophagic activity by dogs on the remains of prey killed by wild predators. Feral dogs can also be a threat to sympatric wild carnivores by transmitting diseases such as rabies and canine parvovirus, since most feral dogs are unvaccinated and are common disease reservoirs or vectors (Funk *et al.,* 2001; Fiorello *et al.,* 2006). In fact, Brickner (2003) remark that in the future, could be an emerging of new infections that will affect endangered wildlife, since the populations of feral dogs are becoming larger and with more intense contact with wildlife. Another negative effect from feral dogs is the hybridization with their wild relatives, not only grey wolves but also other canid species, such as Ethiopian wolves, coyotes (*Canis latrans*) and golden jackals (*Canis aureus*), which may represent a major threat to populations of these wild canids (Gottelli *et al.,* 1994; Adams *et al.,* 2003; Godinho *et al.,* 2011; Galov *et al.,* 2015). This condition can cause the spread of ‘domestication genes’ into wild canid populations, disrupting local adaptations and/or intensifying genetic homogenization, ultimately conducting to the extinction of wild endangered canids through introgressive hybridization (Rhymer & Simberloff 1996).

1.5. Portugal as a case study

Portugal has a human dominated landscape where an endangered wolf population persists in areas with high abundance of free-ranging dogs, becoming an adequate case study to address ecological interactions between wolves and dogs. In Portugal, the Iberian wolf (*Canis lupus signatus*) is listed as “Endangered” in the Portuguese Red Data and fully protected by specific legislation since 1988 (Queiroz *et al*., 2005). According to the last national survey conducted in 2002/2003, the wolf population in Portugal was estimated in about 300 individuals, comprising 65 breeding packs (Pimenta *et al.,* 2005). Currently, the main and most stable populations of wolves are located at north of the Douro river, where a high number of breeding packs occur with geographical continuity with other Iberian populations (Figure 1). Conversely, in the region south of the Douro river persists a small and isolated wolf population, composed of less than 10 packs with low breeding rates, high degree of fragmentation, low genetic diversity and isolated from the remaining Iberian populations, facing a serious risk of extinction (Grilo *et al.,* 2002a; Grilo *et al.,* 2004; Pimenta *et al.,* 2005; Silva *et al.,* 2018). Recently, Silva *et al.* (2018) identified this subpopulation as a distinct genetic clusters from other wolf subpopulations in Iberian Peninsula showing low levels of admixture, suggesting its isolation by Douro river, a large natural barrier to wolf movement with significant anthropogenic development along both banks. Besides, this population at south Douro river persists in a strongly humanized landscape, where domestic animals (e.g. livestock and poultry) are the main food resource (Álvares 2015, Torres & Fonseca 2016). The occurrence of domestic dogs is also considered a main threat affecting this subpopulation, namely due to hybridization with a confirmed event already documented (Torres *et al.,* 2017) and the transmission of diseases with potential demographic impacts on wolves, such as canine distemper virus and a novel bocavirus (Conceição-Neto *et al.,* 2017).

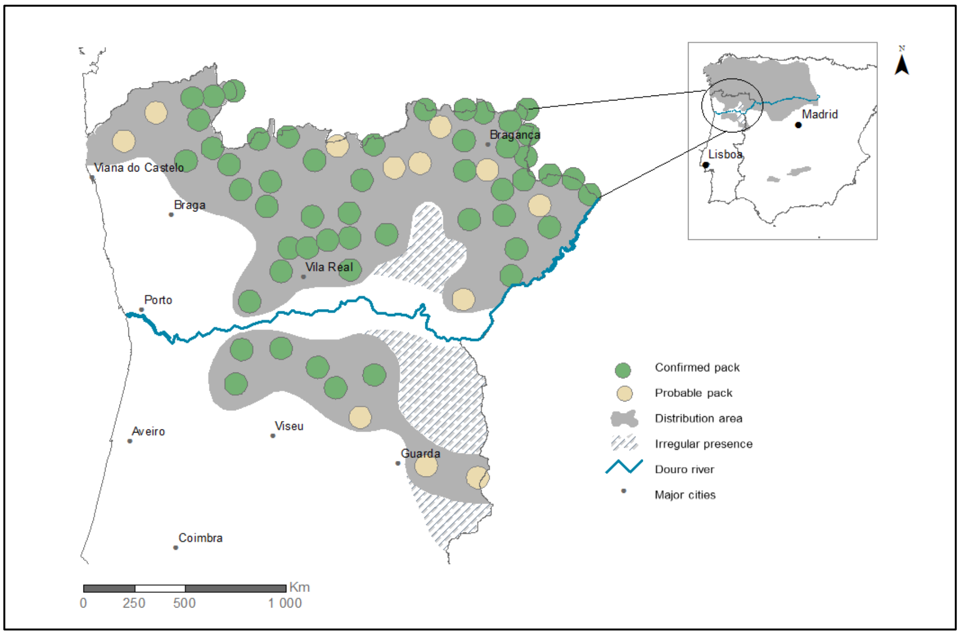


Figure 1 - Wolf distribution in Portugal, representing the main populations and the location of confirmed and probable packs, according to the last National Wolf Census conducted in 2002/2003 (Pimenta *et al.,* 2005).

According to the action plan for the conservation of the Iberian wolf in Portugal, the existence of stray and feral dogs is considered as one of the main threats to the survival of this large carnivore at national level, rendering the need for specific studies to assess competition, predation or hybridization between these two canids (Álvares *et al.,* 2015). In healthy and stable wolf populations the presence of free-ranging dogs does not seem to pose a major threat, as dog consumption by wolves can be relatively frequent (Brito, 2017). However, in reduced wolf population, as the case of in south of Douro river, the widespread presence of feral and/or stray dogs can become a conservation concern due to competition for food and refuge, as well as the transmission of diseases. Another negative factor for wolf conservation, is the predation that dogs exert on domestic animals. In fact, among local people, there is a general scepticism about dogs being able to attack livestock and the predator responsible for an attack is usually attributed to the wolf, increasing the conflict and constraining the necessary conditions for wolf conservation.

A study conducted in late 1990s assessed the population status of stray dogs in Portugal, particularly within wolf range, using questionnaires to hunters, farmers, livestock producers and other relevant authorities (Ribeiro, 1996). This approach revealed a widespread presence of stray dogs throughout Portugal, being reported in 95% of all municipalities of the country, and with higher incidence in several areas within wolf range, particularly at south of Douro river (Figure 2). The main reported causes of the existence of stray dogs were due to the abandonment by hunters (32.9%) during and after the hunting season, and by owners (30.7%). Dog attacks on domestic animals were also reported throughout the territory, with higher incidence in the municipalities located in central and south Portugal, and involving mostly sheep, goats and poultry, while attacks on cattle, horses and asinines were much less frequent (Ribeiro, 1996; Figure 3). The use of waste dumps as a food source was reported in 91% of the municipalities and seemed to play an important role in the survival of stray dogs. Focusing only the regions of north and central Portugal, a similar study (Cortez *et al.,* 2005), reported similar results in terms of stray dogs' occurrence, origin and attacks on domestic animals. Both studies from Ribeiro (1996) and Cortez et al. (2005) provided valuable insights on the ecological, economic, and public health implications related to stray dogs' occurrence in Portugal, despite a poor management actions on this issue conducted so far by the responsible authorities.

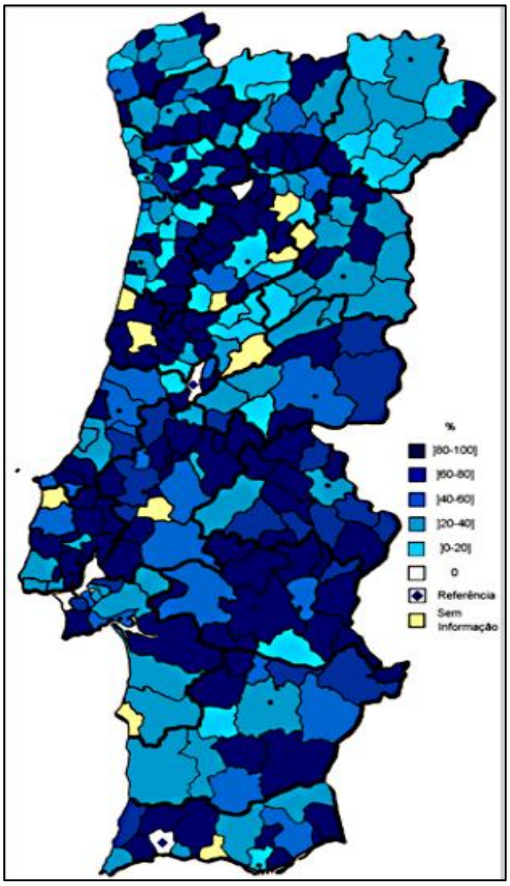


Figure 2 - Distribution of stray dogs in Portugal, per municipality. Blue gradient in each municipality is related to the percentage of parishes, by municipality, with a positive reply to the questionnaire (Source: Ribeiro *et al.,* 1996).

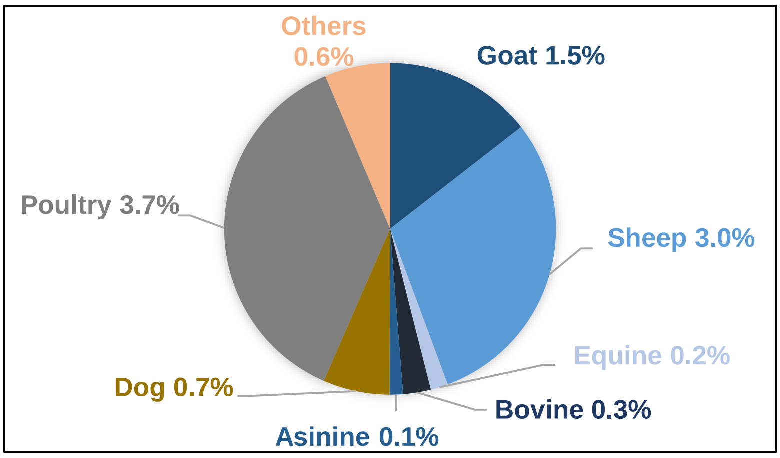


Figure 3 - Domestic animals reported to be attacked by stray dogs in Portugal (Adapted from: Ribeiro *et al.,* 1996).

Regarding to Portuguese legislation on dogs, the Law 8/2017 establishes a legal status of animals, recognizing their nature as living beings endowed with sensitivity and punishing the abandonment given its consequences for public health and safety as well as animal welfare (decree-law 82/2019). This legislation also by promotes responsible detention by owners, including, among other obligations, the identification and registration of pet dogs with an electronic marking system (SIAC: Sistema de Informação de Animais de Companhia) as well as compliance with legal, sanitary and animal welfare parameters. In 2016 a new legislation was approved (law 27/2016) that prohibits the killing of stray dogs as a way of population control, focusing instead on sterilization and measures for the establishment of a network of official collection facilities for stray dogs. Nevertheless, there are increasing reports on the overcrowding of dog kennels as well as the lack of adequate official collection facilities for dogs in many municipalities (Tomás, 2019). Consequently, the legislation forbidding the lethal control of dogs seem to have led, indirectly, to a higher number of stray dogs roaming freely.

Although the population size and impact of free-ranging dogs in Portugal is currently unknown, large dog packs showing aggressive behaviour towards people and attacking livestock have been widely reported by social media, including in areas within wolf range located in northwest (e.g. Viana do Castelo; Jornal Público 2020a) and south of Douro river (e.g. Moimenta da Beira; Jornal Público 2020b). However, despite the strong evidence of wide numbers of free-ranging dogs within wolf range, no studies have focused ecological interactions between these two canids in Portugal. Therefore, there is the urgent need for local studies focusing population estimates and ecological traits of free-ranging dogs as well as their niche overlap with wolves, in terms of space, time and food resources.

1.6. Goals and hypothesis

Considering the current lack of knowledge, the main goal of this study is to address the ecological interactions between domestic dogs and wolves, by assessing for both species population estimates as well as spatial, temporal, and trophic niche overlap in a human-dominated landscape, located in the Portuguese area of wolf occurrence at south of Douro river. To achieve this main goal, information will be obtained based on genetically identified scats, camera trapping and questionnaires to local institutions (municipalities, veterinarians) in order to assess populational, spatial, temporal and trophic patterns in wolves and free-ranging dogs.

To characterize wolf-dog interactions, this study will focus on three main ecological traits: i) Population estimates and Spatial niche; ii) Temporal niche, and iii) Trophic niche.

To investigate **population estimates and spatial niche** of wolves and dogs, this study aims to target the following specific goals and procedures:

1. Estimate abundance of wolves and free-ranging domestic dogs in all study area and per each wolf pack territory, based on genetic individual identifications of scats, questionnaires to local institutions and genetic ID of sampled dogs.
2. Evaluate space use and habitat selection in wolves and free-ranging domestic dogs, based on scats with individual genetic identification.

Based on previous studies on population size and spatial ecology of both canids, we hypothesize that: i) free-ranging dogs are more abundant than wolves (Wierzbowska *et al.,* 2016); ii) free-ranging dogs are highly dependent on human proximity while also using areas included in wolf pack territories (Boitani & Ciucci, 1995); iii) a wide spatial overlap of dogs in wolf territories (Wierzbowska *et al.,* 2016) but a spatial segregation of dogs in wolf breeding sites (Vanak *et al.,* 2014); iv) wolves use areas less disturbed by humans characterized by higher altitude and far from human settlements/activities (Mech, 1989; Theuerkauf *et al.,* 2003b; Capitani *et al.,* 2006; Habib & Kumar, 2007; Person & Russell, 2009; Ahmadi *et al.,* 2014; Iliopoulos *et al.,* 2014; Sazatornil *et al.,* 2016).

To investigate **temporal niche** of wolves and dogs, this study aims to target the following specific goal and procedure:

3. Determine patterns of circadian activity between wolves and dogs using camera trapping data on wolf breeding areas.

Based on previous studies on activity patterns of both canids, we hypothesize that: i) wolf activity will be mostly crepuscular and nocturnal (Ciucci *et al.,* 1997; Theuerkauf *et al.,* 2003c) while dogs will be more active during daytime (Boitani, 1983).

To investigate **trophic niche** of wolves and dogs, this study aims to target the following specific goal and procedure:

4. Assess the diet and trophic overlap between wolves and free-ranging dogs based on the analysis of scats genetically validated, and considering geographical (all

study area and per each wolf pack) and temporal variation (per each season and sampling year).

Based on available knowledge on trophic ecology of both canids, we hypothesize that: i) wolves and free-ranging dogs feed on the same resources, therefore, exhibiting a high trophic niche overlap (Wierzbowska *et al.,* 2016); ii) free-ranging dogs feed on livestock obtained mostly from carcasses in dumpsites and wolf kill remains (Butler & du Toit, 2002; Vanak *et al.,* 2014, Wierzbowska *et al.,* 2016).

These results are expected to provide important insights on wolf-dog interactions and their niche overlap, allowing a deeper knowledge on a topic with worldwide implications as well as the scientific basis to support effective management for wolf conservation in human-dominated landscapes.

2. Study Area

The study area covers approximately 850 km², encompassing the territory of three wolf packs (Lapa, Leomil and Trancoso) belonging to the South Douro river wolf subpopulation in Portugal (Álvares *et al.,* 2015; Serronha *et al.,* 2019) (Figure 4). This area comprises the districts of Viseu and Guarda (Central Portugal), including the municipalities of Aguiar da Beira, Castro Daire, Meda, Moimenta da Beira, Penedono, Sátão, Sernancelhe, Tarouca, Trancoso and Vila Nova de Paiva. The study area has been sampled continuously since 2006 in the scope of on-going projects for wolf monitoring conducted by CIBIO (Research Centre of Biodiversity and Genetic Resources, University of Porto)/ACHLI (Associação de Conservação do Habitat do Lobo Ibérico) in the context of Environmental Impact Assessments for windfarm development, and by resorting to a multi-methodological approach based on sign surveys, camera-trapping and non-invasive genetics (Serronha *et al.,* 2019). Sampling design for scat detection was based on a network of transects surveyed monthly and evenly distributed across 34 UTM grid of 5x5 km (Figure 5). This area was delimitated by considering the average size of pack territories in Portugal, ranging 150 to 300 km² (Roque *et al.,* 2011) and includes several human settlements and other infrastructures (e.g. intensive farms for poultry, rabbit or swine production) where the presence of domestic dogs is frequent.

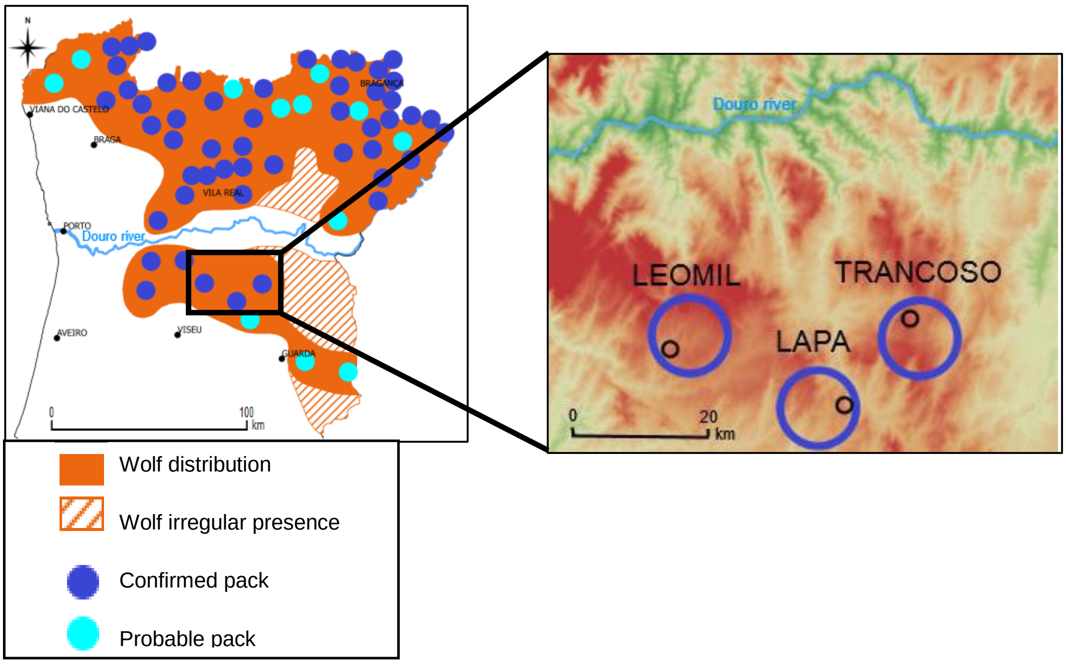


Figure 4 - Wolf range in Portugal including known packs according to the last National Wolf Census conducted in 2002/2003 (Pimenta *et al.,* 2005), the location of the study area including the three surveyed packs and respective breeding sites (black circles).

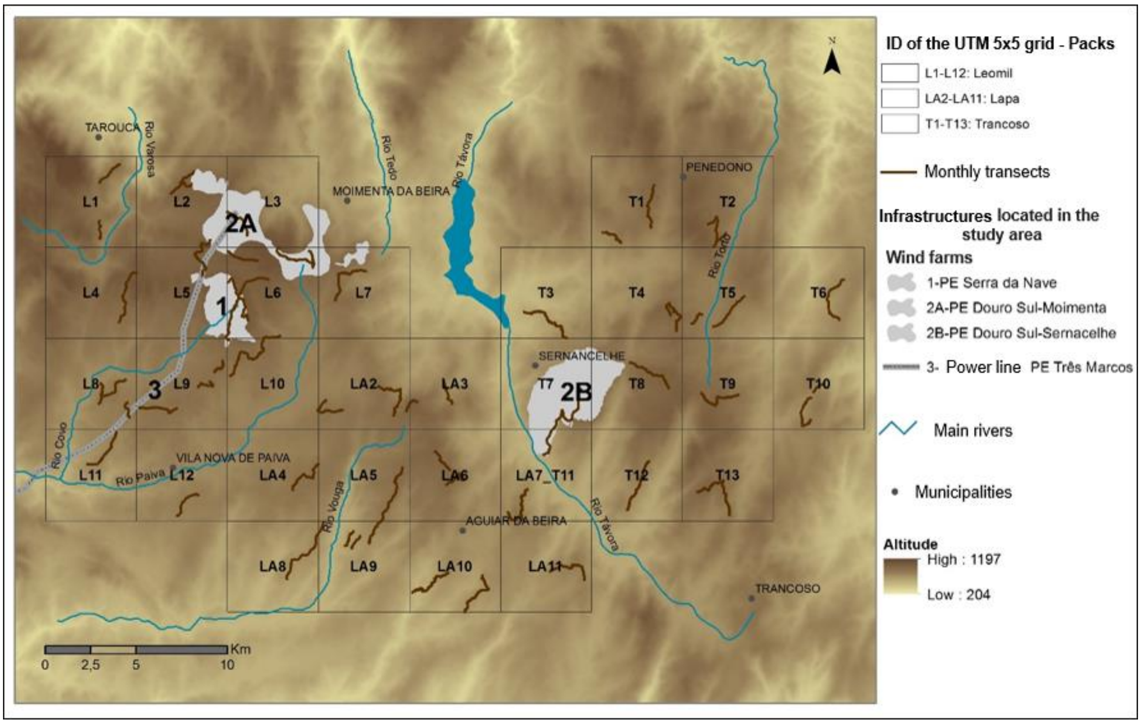


Figure 5 - Location of the sampling area for wolf monitoring in South of Douro river (in the scope of on-going projects from CIBIO/ACHLI), including monthly transects for scat detection distributed in a UTM 5x5km grid assigned to each pack, and which comprise the study area and available data considered in this study (source: Serronha *et al.,* 2019).

The study area is a mountainous region with low slopes, with average altitudes ranging 700-800m and a maximum altitude of 1011m. It contains the mountain ranges of Leomil, Lapa, Sirigo and several hills located between Penedono and Trancoso, mostly composed of volcanic rocks such as granite (Pena & Cabral, 1996). The main rivers in the region are Paiva, Tedo, Torto, Vouga and Távora (Serronha *et al.,* 2019). The average daily temperature is 13.6°C and 10.9°C in Viseu and Guarda district, respectively, and the average total rainfall per year reaches 882 mm in Guarda and 1170mm in Viseu (IPMA, 2020). Vegetation cover is dominated by scrublands of *Erica* spp. and *Cytisus* spp., and production forests composed of *Pinus pinaster* and *Eucalyptus globulus*. The native forest is confined to small patches mostly along river valleys and dominated by *Quercus robur* below 600m and *Quercus pyrenaica* above this altitude (Pena & Cabral, 1996). It is a predominantly rural region, with a human population density of 32 inhabitants/km² (INE, 2011). The landscape is very heterogeneous and human-dominated, characterized by a combination of human settlements, agricultural land, scrublands and forest plantations. Human settlements and agricultural land are predominant at lower altitudes, whereas scrublands and forests are more common in more mountainous areas. Human activities are present throughout wolf range such as livestock production, hunting, and infrastructure development (e.g., wind farms, quarries for stone extraction and roads), being common practices even in the most remote areas (Serronha *et al.,* 2019). Regarding livestock production, there are extensive grazing of small ruminants (sheep and goats) during the day, frequently with the presence of a shepherd and/or mid-size dogs, intensive production or confined grazing of cattle and horses as well as the presence of several intensive production farms of rabbit, pigs, and poultry (see Table 1; Torres *et al.,* 2015a; personal observations).

Table 1 - Number of individuals (N) and respective percentage of each domestic prey in the study area, located at South Douro region based on available information from 2009 (Source: INE, 2011).

Domestic Prey	N	%
Poultry	347974	93.7
Rabbits	16439	4.4
Cattle	2298	0.6
Sheep	1983	0.5
Goats	1978	0.5
Pigs	698	0.2
Horses	122	0.03
Total	**371492**	**100**

There is no available information on the population size of domestic dogs in the study area, although hunting activities and livestock production often have associated working dogs (personal observations) and previous studies reported the widespread presence of stray and feral dogs (Ribeiro, 1996; Cortez *et al.,* 2005). Furthermore, based on the sign surveys conducted in the scope of on-going projects for wolf monitoring in the study area, the percentage of detected scats, originally attributed to wolves but genetically validated as dogs, was 58% and 29% in 2017 and 2018, respectively (Serronha *et al.,* 2018; Serronha *et al.,* 2019), suggesting the widespread occurrence of free-ranging dogs within wolf range.

Potential wild prey for wolves in the study area are only represented by lagomorphs, such as rabbit (*Oryctolagus cuniculus*) and hare (*Lepus europaeus*), as well as wild ungulates. Wild ungulates are mostly represented by the wild boar (*Sus scrofa*), which is common and widespread. Although there is no published information available on wild boar abundances, according to CONFAGRI (2009) and Terras de Sicó (2017), seems to occur at high densities in all continental Portugal, estimated in 10 individuals/100 ha. The roe deer (*Capreolus capreolus*) has been reintroduced in the region but its presence and abundance are still limited inside the study area (Torres *et al*., 2015b). This area also harbours a diverse community of mesocarnivores, such as the common genet (*Genetta genetta*), beech marten (*Martes foina*), badger (*Meles meles*) and red fox (*Vulpes vulpes*), along with the largest carnivore, the Iberian wolf (Bencatel *et al.,* 2019).

The south Douro's wolf subpopulation, estimated in less than 8 breeding packs (Álvares *et al.,* 2015), had been identified as one of the few in Europe on the verge of local extinction (Boitani & Ciucci, 2009). Indeed, local habitat conditions appeared to be poor (Grilo *et al.,* 2002a) and local recruitment seems to be restricted due to low fecundity (Álvares *et al.,* 2015) and negligible immigration (Silva *et al.,* 2018). Based on the minimum count of individual genetic profiles obtained from scats (used in the analysis of this study: see chapter 3.1.), the estimated wolf population size in our study area during 2018 was a total of 12 individuals (7 males and 5 females), one of them corresponds to a juvenile female killed by traffic collision (Serronha *et al.,* 2019). Considering each pack, in 2018 Leomil pack was estimated in 8 wolves (5 males and 3 females), Lapa pack in 2 individuals (1 male and 1 female); and Trancoso pack also in 2 individuals (1 male and 1 female) (Serronha *et al.,* 2019). A brief description for each wolf pack included in our study area follows.

Lapa pack

The territory of this pack occupies mostly Serra da Lapa, located southwest of Sernancelhe and includes four main activity centers, that have been used as breeding sites: "Lapa", "Picoto", "Cabeça Cimeira" and "Penedo do Homem" (Serronha *et al.,* 2019; Figure 6). Lapa pack was first detected in a national survey conducted in 1994 (Pimenta *et al*., 2005) and has already been the target of ecological studies involving population estimates, the capture and monitoring of individuals by radiotelemetry and diet analysis (Bastos, 2001; Quaresma, 2002; Grilo *et al.,* 2002a). Over the last 20 years of monitoring, this pack showed a small group size (2-3 adults) and a low breeding success (30%) (Serronha *et al.,* 2019). During the time period included in this study (2014 to 2018), this pack reproduced during 2014 and 2016 (Roque *et al*. 2015; Roque *et al*. 2018; Serronha *et al.,* 2019).

Figure 6 - Breeding area from Lapa pack, Aguiar da Beira (© Ricardo Brandão)

Leomil pack

The territory of this pack occupies Serra de Leomil, located between Vila Nova de Paiva and Moimenta da Beira, and includes three main activity centers, that have been used as breeding sites: “Corgo da Cerca”, “Corga do Redondelo” and “Rio Covo” (Serronha *et al.,* 2019; Figure 7). Leomil pack was first detected in a national survey conducted in 1994 (Pimenta *et al*., 2005) and is one of the most studied in the wolf subpopulation at south of the Douro River, having already several individuals monitored by telemetry and different assessments of diet analysis (Alexandre *et al.,* 2000; Bastos, 2001; Quaresma, 2002; Grilo *et al.,* 2002a; Roque *et al.,* 2005; Sobral, 2006; Pinto, 2008; Casimiro, 2017). Over the last 20 years of monitoring, this pack showed a reasonable group size (3 to 5 adults) and a breeding success of 43%, being considered the most stable of the three studied packs (Serronha *et al.,* 2019). In the current study time frame (2014 to 2018), this pack only reproduced in 2014 and 2018 (Roque *et al*. 2015; Serronha *et al.,* 2019).

Figure 7 - Breeding area from Leomil pack, Vila Nova de Paiva (© Barbara Martí)

Trancoso pack

The territory of this pack occupies the mountainous areas located north of Trancoso and east of Sernancelhe, and includes three main activity centers, that have been used as breeding sites: "Medonho", "Cabeças" and "Lagar" (Serronha *et al.,* 2019; Figure 8). Trancoso pack was also detected in a national survey conducted in 1994 (Pimenta *et al*., 2005) and, together with Leomil and Lapa packs, has been also the target of ecological studies involving population estimates, radiotelemetry, diet analysis and quantification of predatory impact on livestock (Bastos, 2001; Grilo *et al.,* 2002a; Quaresma, 2002; Pereira, 2003). Over the last 20 years of monitoring, this pack showed a small group size (2-3 adults) and a very low breeding success (14%) (Serronha *et al.,* 2019). Considering the time period included in this study (2014 to 2018), this pack reproduced only in 2015 (Roque *et al*., 2017; Serronha *et al.,* 2019).

Figure 8 - Breeding area from Trancoso pack, Sernancelhe (© Patrícia Gil)

3. Methodology

This study used different methodological approaches and datasets in order to estimate population size of wolves and domestic dogs, as well as, to compare distinct measures of their ecological niche: spatial, temporal and trophic.

Overall, the population estimations and habitat determinants (spatial niche) of wolves and dogs were assessed by capture-recapture models based on scats genetically identified, obtained from monthly prospections in transects between 2014 and 2018. Also, information was gathered through questionnaires to local institutions (municipalities and veterinarians). Furthermore, in order to get additional data on the characteristics of the studied dogs, saliva samples from pet dogs were also collected in the study area during 2020, in order to find matches with the individuals that were genetically identified on scats. Regarding the analysis of circadian activity (temporal niche), data were obtained using camera trapping in wolf breeding sites during 2018. Diet analysis (trophic niche) was based on the identification of prey remains in scats genetically identified and collected between 2014 and 2018.

In the next sections, the methodological procedures for each parameter are described with further detail.

3.1. Population estimates, habitat determinants and activity patterns

Data source and field sampling

The genetic individual identification of scats was obtained from monthly scat detection in transects conducted between 2014 and 2018. Scat samples from wolves and dogs were already available and genetically analysed in the scope of on-going monitoring projects conducted by CIBIO and financed by ACHLI.

Scats visually and morphologically attributed to wolves were collected along transects (trails and unpaved roads), selecting areas frequently used by wolves to deposit faeces for territorial scent-marking, particularly crossroads (Barja *et al.,* 2005). Transects were sampled monthly by a vehicle at low speed (<10 km/h) to allow detection of potential scats and sampled by foot in approximately 100 m for each side of a crossroad. Scats were collected in the field if considered as being from wolves according to different criteria, such as shape, size, scent and composition of scats (Sanz & Domínguez, 2015), the proximity to known core areas of breeding packs (Pimenta *et al.,* 2005) and the level of human presence or activity to reduce the possibility to find scats from domestic dogs. In case of doubt about taxonomic affiliation to other canid species (e.g. red fox and domestic dog), the scat was disregarded, although posterior genetic analysis confirmed several scats as still being from dogs. Each detected scat attributed to wolf was collected and stored in sterilized tubes labelled with an ID and preserved in 96% ethanol. A small portion from each scat was genetically analysed for species and individual molecular identification, in CIBIO's lab. DNA from scats was extracted according to Frantz *et al.,* (2003) after the GuSCN silica method. Species identification was assessed through the amplification of an approximately 425 bp sized fragmented of the mtDNA control region, and then samples with wolf and dog mtDNA were genotyped for a set of 13 microsatellites for individual identification (for additional specifics on the molecular procedures for this analysis see: Nakamura *et al.,* 2017). The exact location of each collected scat was recorded with the application MapIt 39 (version 5.0.5), a tool developed for the collection of data for Geographic Information Systems (GIS), based on Global Positioning Systems (GPS).

For the analyses of the activity patterns of wolves and dogs, available records obtained by camera trapping conducted were used in all three packs, during 2018. Photographic trapping stations involved the placement of cameras (KeepGuard ® KG 780 NV) with automatic firing, directed to the detection of wolves in traditional breeding areas and other wolf core areas with strong evidences of its presence (Serronha *et al.,* 2019). Remote cameras are activated by motion sensors, supported by visible flash lamps. The cameras were placed on sites with difficult access and visualization by humans (under stones, for example) and fastened by padlocks to prevent robberies. In some of the locations were placed bait (Coyote or Call) to attract the animals. During the sampling period, motion was detected by the sensor on every occasion, the camera initiated a video recording of 30 or 40 seconds or took three instant photos with 1 second interval. All recorded videos and photos were visualized and organized in an Excel database, including type of record (e.g. target species or other), date, initial time and location. The types of records were animal species, including wolves and dogs, or sources of direct or indirect human disturbance. Regarding to the target canids, for records of wolves was noted the number of individuals, sex (if possible) and the number of pups, while for dogs' other additional information was noted, such as association with humans, presence of collar, type of activity (e.g. livestock guarding dogs, hunting dogs, pet dogs) and also, morphological and phenotypic characteristics to allow individual identification through camera trap.

Furthermore, other additional approaches were used to better evaluate the population size and characteristics of the domestic dogs in the study area. First, was conducted field work in February and June 2020 to sample and characterize domestic dogs in the study area, in order to achieve a match with individuals identified either by non-invasive genetics or camera trapping. Dogs were selected if known to wander within wolf range, and a saliva sample was collected for posterior identification of the genetic profile. DNA was isolated from the saliva samples, preserved in 96% ethanol using the commercial QIAamp DNA Micro Kit (QIAGEN) after an overnight prewash in lyses solution and following the manufacturer's instructions. To monitor for potential DNA contaminations, negative controls were included throughout the entire process (Nakamura *et al.,* 2017). Furthermore, a semi-structured questionnaire was performed to the owner of each sampled dog to assess traits related to sex, age, size, breed, type of activity, management conditions, etc. It was possible to collect 15 samples (14 samples of saliva and 1 sample of hair) from domestic dogs, which were genetically analysed for individual identification following the same procedure described above for scats. Second, a request (via e-mail) for specific information was made to the Municipalities and local veterinarians in the study area. We requested data regarding the number of domestic dogs registered by municipal veterinarians; the number of observed stray dogs (estimate); occurrence of attacks by dogs on cattle or persons and number of captures of abandoned dogs. Of the 10 municipalities surveyed, 6 (60%) responded and provided the requested data for 2019 and early 2020.

Data analysis

Abundance estimates were based on the minimum number of wolves and dogs in all study area and per each pack territory. Estimates for wolves were based on intensive monitoring by different methods (e.g. howling surveys, camera-trapping, genetic individual identification of scats) while estimates for domestic dogs (including registered pet dogs and stray dogs) were based on intensive monitoring (e.g. camera-trapping, genetic individual identification of scats) and information gathered from questionnaires to Municipalities and local veterinarians.

Abundance, density, probability of detection and estimation of wolves and dogs was calculated through Spatial Capture-Recapture methods (SCR) applied to individual genetic identification of scats. The spatial interactions were made based in a subsequent correlational analysis, with the outputs of each of the models made independently. The standard SCR model was assumed that a population of *N* individual was sampled with an array of *J* traps during *K* occasions producing an encounter of *n* individuals (Royle *et al.,* 2013). Each individual in the population is expected to have an activity center *s*, characterized by the spatial location of individuals during the sampling period (Royle *et al.,* 2013). The activity center of the individual *i* was represented by the respective coordinates, and its movement were distributed according to some probability (Royle *et al.,* 2013). The distribution of activity centers is assumed to be uniform where the density of points is constant (see Royle *et al.,* 2018). Spatial encounter histories of individuals are built with encounter frequencies *y (i,j,k),* assumed to be Bernoulli outcomes (Fuller *et al.,* 2016) where the probability of detecting an individual at site x is assumed a function of the Euclidean distance between the individual's activity center, *s*, and *x* (Royle *et al.,* 2013) and we represented it by the half-normal distribution (see Royle *et al.,* 2018, Sutherland *et al.,* 2019).

SCR estimates were derived from the "oSCR" package (Sutherland *et al.,* 2019) on R software. Based on Sutherland *et al.,* (2019), oSCR requires a Trap Deployment File (TDF) comprising the name and coordinates of each scat identification associated to the 58 transects, trapping effort and respective covariates. The sampling occasion was one month and the sampling area was considered as a buffer equivalent to the radius of half of the longest transect. For the different covariates, was taken into account the characteristics of the study area and biological traits of the two target species. For wolves, the covariates were the distance to the nearest human build up (data acquired through a database from the European Space Agency for mapping the European Human Settlement with detail of 10x10 m), distance to wolf breeding sites and mean altitude. Dogs had the same covariates, except for the altitude that has been removed due to the fact that biologically we would not expect greater abundance of dogs in areas with higher altitude but rather in areas where there was greater human presence. The other object required for SCR in oSCR is the Encounter Data File (EDF), which contains information on the numeric session (2014 through 2018), the individual identification of scats, the sampling occasion (12 months) when the individual was encountered and sex (Sutherland *et al.,* 2019).

The state-space object was built in oSCR by imposing a buffer on transect centroids equivalent to 1.5 the mean maximum distance moved (mmdm) across all identified individuals, as assessed by genetically profiled scats. A 2x2km resolution pixel was selected for spatial inference.

SCR models required the joint estimation of 3 parameters – Density (*D*), Baseline detection probability (*p*) and spatial scale parameter (σ), accommodated in two sub-models: the detection and the state model. For the state model, variables that might affect the biological process were chosen. For the state model, variables affecting wolf detectability were included as baseline detection covariates, whereas factors affecting rate of spatial decrease in detection probability were considered. The combinations of the effects were: ~1 for no effects; ~session for year-specific effects; ~Tr for assessing potential trends; ~sex for sex effects. Then all model covariates were combined and the best was selected as the one with the lowest AIC. Since several models fulfilled the ΔAICc<2 criterion for the most supported hypothesis, we adopted a model averaging procedure to compute the average coefficients of covariates included in the best models and their corresponding 95% confidence intervals (CI 95%) (Burnham & Anderson 2002).

Once the model is selected, the predictions about density, baseline detection probability and spatial scale of detection could be realized, comprising the realization of the SCR model implemented.

To determine the circadian activity of both target species based on camera-trapping data, all time periods were processed continuously and only independent records that are separated temporally in 30 minutes were used and calculated. It was required the package “activity” (Rowcliffe, 2019) in R software. This package presents tools for plotting activity distributions, the overall level of activity with error and allows for statistical comparison distributions through bootstrapping using the core function “fitact” (Rowcliffe *et al.,* 2014). This core function fits kernel density to radian time-of-day data and estimates activity level from this distribution, being made a bootstrap with 500 repetitions of the data in order to obtain the confidence limits and weights of the distribution. The temporal segregation between the two species in each sampling season was evaluated by pairwise comparisons of their activity patterns, according to the procedure followed by Monterroso *et al.,* (2016b), estimating the coefficient of overlap (Δ1) through the Mardia-Watson-Wheeler test to relate the distribution of detections across the daily cycle between both species.

3.2. Diet composition

Data source and field sampling

Diet composition of wolf and dogs was based on the analysis of scats genetically validated collected between 2014 and 2018. The sampling period including several years was selected in order to obtain an equitable sample size for both species, by fulfilling the following requirements: i) total sample size >90 for each target species to allow reliable results such as the detection of prey items poorly represented (Reynolds & Aebischer, 1991; Trites & Joy, 2005); ii) spatial representativeness of the target packs; and iii) uniform sample sizes throughout seasons. Details related to field sampling for scat detection along transects, collection of samples and procedures for genetic analysis were described previously in subchapter 3.1..

Laboratory procedures

The collected scats genetically confirmed as wolves or domestic dogs, were analysed in laboratory to assess diet composition in each species. The micro and macro-components of each scat were separated by thorough washing in a sieve with a mesh size of 0.5 mm. The microscopic fraction of the scat, composed by soluble particles and fragmented components that pass through the sieve, was discarded assuming it proceeds from food items in the same proportions as the macroscopic remains. All other macro-components remain, retained by the sieve, were oven-dry at a mean temperature of 60 ºC, for 24 hours in order to be further identified in this analysis. The point-frame method was used as a standardized procedure to identify hairs and other macroscopic remains from prey items in each scat sample, following the procedure described in Chamrad and Box (1964), Ciucci *et al.,* (2004) and Casimiro, (2017). This method was proved to be reliable and efficient for wolf diet studies based on scat analysis as it is a consistent alternative to hand and non-systematic separation of the macro-components, allowing better results, a significant reduction in time and effort in processing faecal samples as well as a uniform and reliable detection of all food items (Ciucci *et al.,* 2004). Individual macro-components were separated according to the following categories: hair, bones, cartilage, feathers, vegetal material, mineral material, insects, garbage (plastic and other human materials) and non-identified material.

The hairs were carefully selected in each scat sample, being the basis for identifying the consumed prey through the microscopic examination of their cuticular patter, medulla, and cross-section. Based in the hair particularities of each prey species, the specific *taxon* was identified whenever possible by following the criteria from Debrot *et al.* (1982), Teerink (1991), De Marinis & Asprea (2006), Valente *et al*. (2015) besides consulting a reference collection from CIBIO with hairs from each potential wolf prey in the study area. Remains of feathers were identified microscopically to the taxonomic Order and were compared with material from the reference collection from CIBIO. The basis for their identification was the characteristic of the nodes and internodes, specific for the taxonomic Orders found in the study area (unpublished data from Sara Roque, Grupo Lobo/FLUP). The items considered non-food material were identified macroscopically and their number of occurrences were quantified (Appendix I), although they were not included in the results as a prey item. These items included vestigial hairs from the target species (wolf or dog, assumed from grooming), non-identified material, material ingested intentionally, such as bones and purgative plants, and material considered to be ingested involuntarily, such as mineral material, plant leaves, insects, and garbage from human's source.

Data analysis

The diet composition of both species was quantified by two indexes commonly used on dietary studies of wolves and other carnivores, but which provide complementary information (Ciucci *et al.,* 1996): the relative Frequency of Occurrence (F.O.) and Consumed Biomass (Biomass). Although F.O. is a very informative approach, can have some associated bias by underestimating large bodied or less consumed prey that can be found in several different scats but originated from one single prey, and overestimating more commonly consumed prey, considering variances in a ratio surface/volume between a small and a large prey (Ciucci *et al.,* 1996). Therefore, the understanding of scat-analysis data to assess the diet of wolves is considered to be significantly improved by comparing results obtained from these two approaches (Ciucci *et al.,* 1996).

The relative F.O. was expressed as an absolute percentage of the number of occurrences of each prey item in relation to the total number of occurrences of all identified prey, allowing a direct knowledge of the relative importance of each prey item in the sample. With the purpose of categorizing the relevance of F.O. for each prey item, values were classified according with Ruprecht (1979) in: i) Basic food resource (F.O. ≥ 20%); ii) Regular food resource (20% > F.O. > 5%); iii) Supplementary food resource (5% ≥ F.O. > 1%); and iv) Occasional food resource (F.O. ≤ 1%).

The percentage of Biomass of each prey class was estimated by the model of Weaver (1993), adjusted from a preceding model from Floyd *et al.,* (1978) and represented through a linear regression: y=0.439+0.008x, where the dependent variable (y) represents the biomass ingested per collected scat and the independent variable (x) represents the mean live weight (kg) of each prey class identified in the scat. Multiplying the value of y by the number of scats in which the corresponding prey was found present, is possible to acquire the total amount of consumed Biomass for each prey item. The identification of consumed adults and juveniles in this study was possible to obtain only for wild boar (*Sus scrofa*) based on physical and microscopic analyse of guarding hairs, which allowed to grossly differentiate their age class as adults and juveniles with less than 6 months (unpublished data from Sara Roque, Grupo Lobo/FLUP). For the other prey classes, were considered average weights of adult individuals from each prey item (Appendix II). For the identification of cat hairs, it was not possible to discriminate with certainty between wildcat (*Felis silvestris*) and domestic cat (*Felis catus*) being considered the mean weight between the two species. Since all identified feathers were from Galliformes, the average weight applied is from domestic chickens raised in intensive farms (Appendix II).

Dietary results from each target species were presented by season, by sampling year, by each pack territory and considering all study area. To evaluate statistical differences between dietary results from each canid species, sampling year and seasonal variation, it was applied a χ^2 test, with a significance level of 5% and Yates correction for 1 degree of freedom (Cochran, 1952). The Yates correction for continuity (Simpson *et al.,* 1960) was used in order to minimize the possible bias of the traditional restrictions respecting the minimum expected frequencies, dictating that the expected frequencies must be superior to 5 (Cochran, 1952; Roscoe & Byars, 1971). Seasons were categorized as: Winter (January 1st to March 31th); Spring (April 1st to June 30th); Summer (July 1st to September 30th) and Autumn (October 1st to December 31th).

We also quantified the level of overlap in the trophic niche of wolves and dogs, by using Pianka's Symmetrical Index (Krebs, 1989):

$$\boldsymbol{N.O.} = \frac{\Sigma(p_{ij} \cdot p_{ik})}{\sqrt{(\Sigma p_{ij2} \cdot \Sigma p_{ik2})}}$$

being p the proportion of a certain prey class i in the carnivore j and carnivore k. The overlap achieves the maximum value of 1 if all prey classes are exploited between target species and attains the minimum value of 0 if there is no common prey between dogs and wolves.

Finally, to infer the feeding behaviour of wolves and dogs related to livestock consumption in the study area (predation vs. scavenging), we compared the proportion between the F.O. of each livestock species in wolf diet and the corresponding number of wolf attacks declared and confirmed by ICNF inside the same area and during the same period between 2014 and 2017. The official data on wolf damages to livestock was not available for 2018 and is important to note that data from 2017 may not be strictly comparable to previous years, due to changes in the compensation system for losses attributed to wolves (V. Pimenta & I. Barroso, ICNF, *pers. com.*).

4. Results

Population estimates and habitat determinants

Overall, we used a total of 606 scats genetically confirmed collected between 2014 and 2018, including 263 scats from wolves and 343 scats from dogs (Figure 9). From these samples were extracted the individual genetic profile resulting in 163 individual genetic profile of dogs and 165 individual genetic profile of wolves.

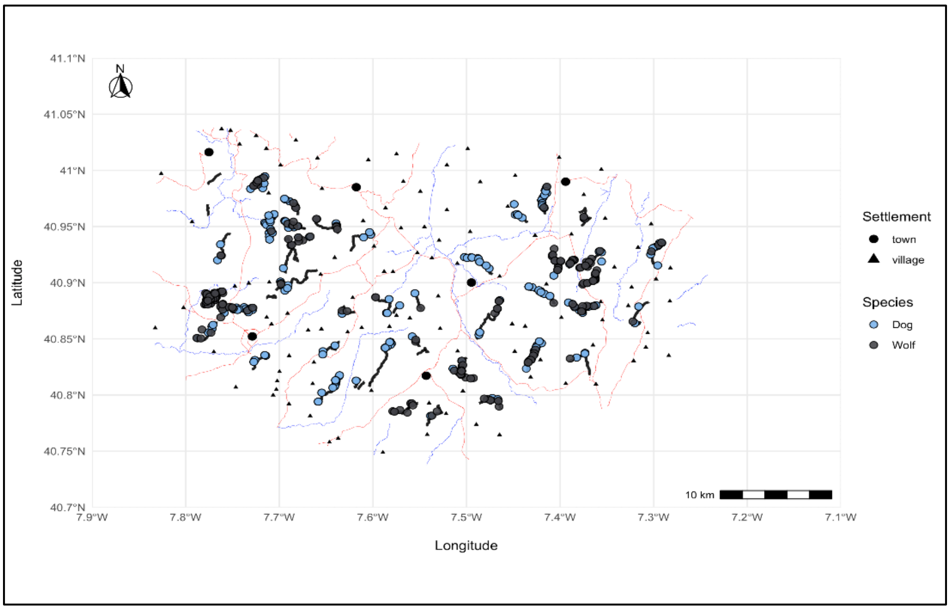


Figure 9 - Location of wolf and dog scats genetically validated.

For both dog and wolf, are presented the top supported models used to evaluate the abundance of these canids in South of Douro (Table 2; see Appendix III).

Table 2 - Top-supported models used to evaluate the density (D~), baseline detection (p~), the spatial scale of detection (sig~) and cost function (asu~) of the Iberian wolf and domestic dog in South of Douro, between 2014 and 2018, considering different covariates (zRBuildUp - distance to the nearest human build up; zdCAct - distance to wolf breeding sites; zAlt - mean altitude) and combinations of the effects (~1 for no effects; ~session for year-specific effects; ~Tr for assessing potential trends; ~sex for sex effects).

Iberian wolf best model	**LogL**	**K**	**AIC**	**dAIC**	**weight**	**CumWt**
D(~zRBuildUp) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.36	12.00	1 622.72	0.00	0.21	0.21
D(~zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.42	12.00	1 622.83	0.11	0.20	0.41
D(~Tr) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.43	12.00	1 622.85	0.14	0.20	0.61
D(~zRBuildUp + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.97	13.00	1 623.94	1.22	0.11	0.72
D(~Tr + zRBuildUp) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.13	13.00	1 624.26	1.54	0.10	0.82
D(~Tr + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.19	13.00	1 624.38	1.66	0.09	0.91
Domestic dog best model						
D(~session + zRBuildUp) p(~session) sig(~1) asu(~1)	814.00	13.00	1 654.01	0.00	0.30	0.30
D(~session) p(~session) sig(~1) asu(~1)	815.27	12.00	1 654.53	0.52	0.23	0.53
D(~session + zRBuildUp + zdCAct) p(~session) sig(~1) asu(~1)	814.00	14.00	1 656.01	2.00	0.11	0.64
D(~session + Tr + zRBuildUp) p(~session) sig(~1) asu(~1)	814.00	14.00	1 656.01	2.00	0.11	0.75

According to the best model, wolf population had a near-stationary trend during the sampling period and was composed by 12 ± 2.44 males and 8 ± 2.24 females (Appendix IV). Conversely, dogs have higher and more irregular values, with males varying between 48 and 216 individuals and females between 38 and 132 individuals (Appendix IV). The year with the highest number of dogs was 2014, followed by a strong decline in numbers during the following years and a new increase in 2016 and 2017.

Inference about the spatial variation on density was made using model-averaged coefficients, so it includes all variables initially considered (Appendix V). For wolves, the variable with more weight was mean altitude with the value of 0.13 ± 0.18 while for dogs was distance to human buildings with 0.11 ± 0.07 (Appendix V).

According to the model set with more support, a slight decline in abundance of the wolf population has been shown over the years, corresponding to 6.6% (Figure 10) as for dog population is also observed a decline of 46.6% over the years (Figure 11). Dogs are much more dispersed in the study area than wolves (Figure 11). Overall, dogs have a much higher density value (in proportion, 25 times more density of dogs than the density of wolves) (Figure 10; Figure 11). Models predicted a higher wolf density at higher altitudes and closer to human build up (Figure 10), while dogs have a higher predicted density at longer distances from human build up and their density does not vary with distance to wolf breeding sites (Figure 11).

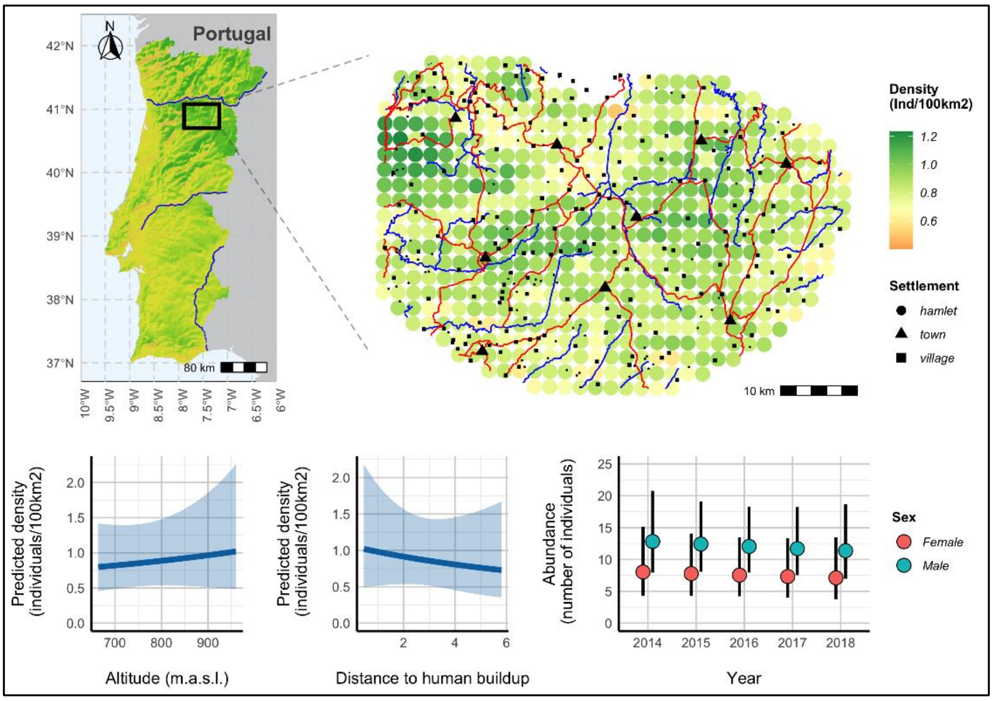


Figure 10 - Map with wolf density in the study area located at south Douro river and graphs with predicted wolf density in relation with altitude, predicted wolf density in relation with distance to human build up and abundance of wolves (including males and females) between 2014-2018. Inset map with location of the study area at a national context. Rivers and streams as blue lines; motorways, main and secondary roads as red lines.

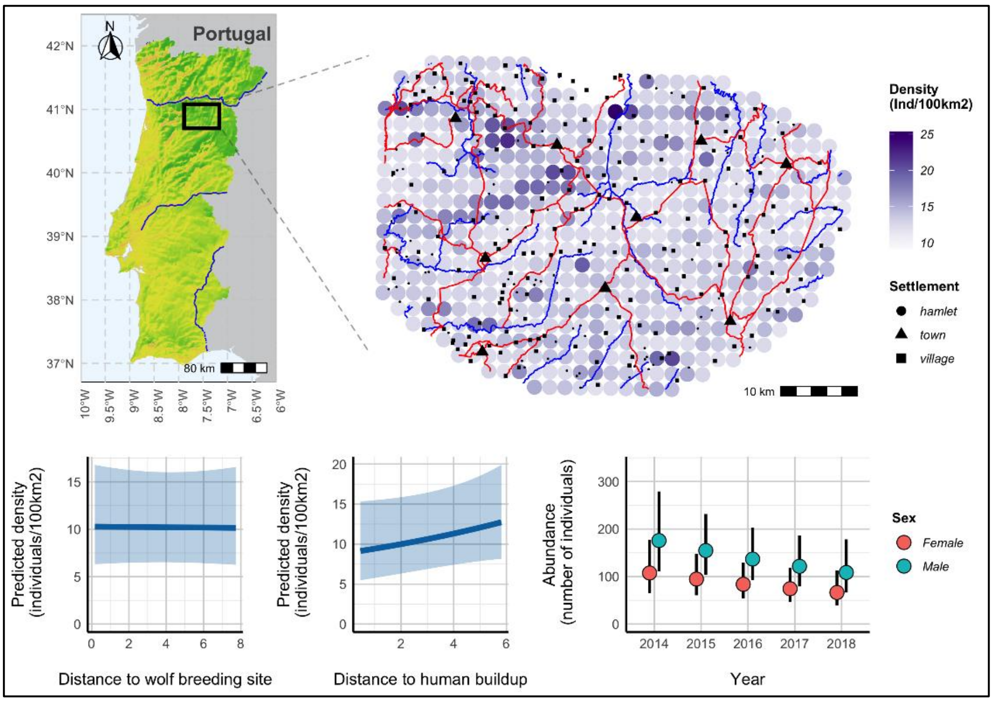
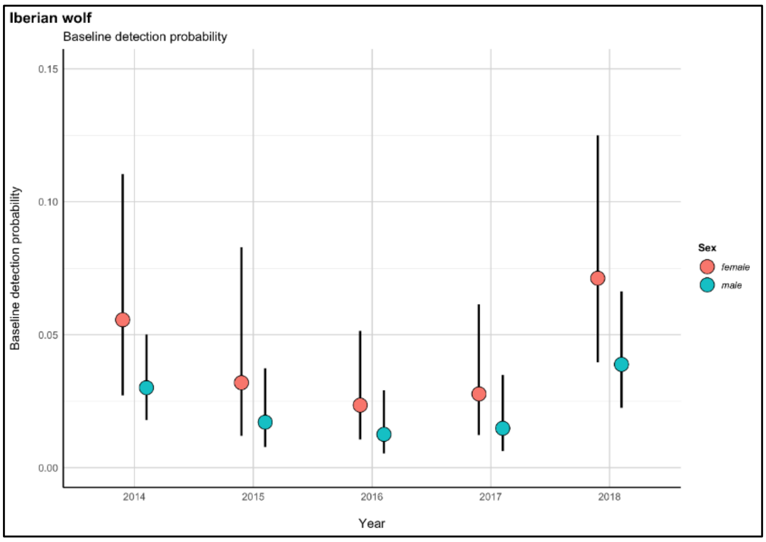


Figure 11 - Map with dog density in the study area located at south Douro river and graphs with predicted dog density in relation with distance to wolf breeding site, predicted dog density in relation with distance to human build up and abundance of dogs (including males and females) between 2014-2018. Inset map with location of the study area at a national context. Rivers and streams as blue lines; motorways, main and secondary roads as red lines.

Regarding the detection rate, for wolves there is a greater detection of females compared to males with a decrease in 2015 and 2016, followed by an increase in the following years, reaching the maximum value in 2018 (Figure 12; Figure 13).



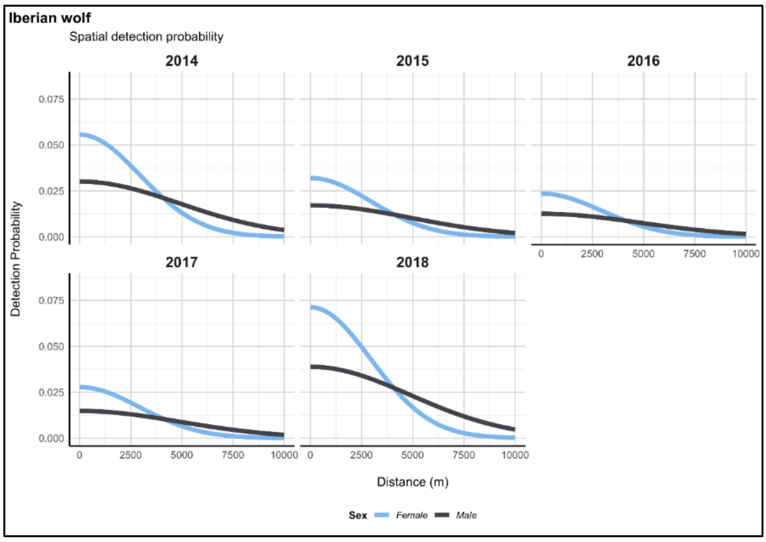
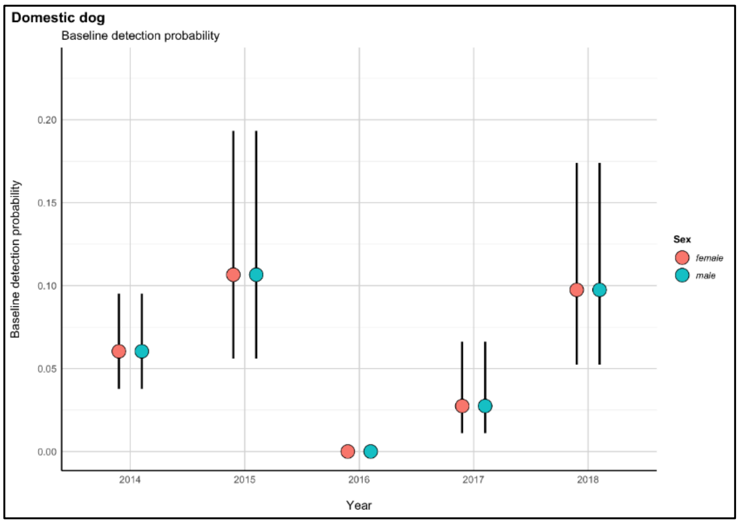


Figure 12 - Baseline detection probability of wolf females and males between 2014 and 2018.

Figure 13 - Spatial detection probability between wolf females and males between 2014 and 2018. The spatial detection probability is calculated from the distance to the target activity center, created by the model (e.g. centroid of each sampling transect).

For dogs, there is no difference in detection probability between males and females. Despite the baseline detection probability is equal between sexes, they show to be more irregular compared to wolf values, with an increase in 2014 and 2015, followed by a severe decrease in 2016, increasing again in 2017 and 2018. (Figure 14; Figure 15).



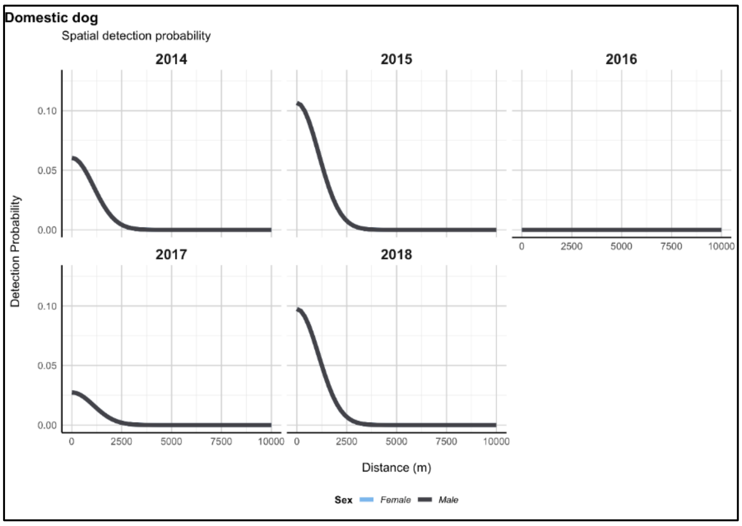


Figure 14 - Baseline detection probability of dog females and males between 2014 and 2018.

Figure 15 - Spatial detection probability between dog females and males between 2014 and 2018. The spatial detection probability is calculated from the distance to the target activity center, created by the model (e.g. centroid

Finally, the relationship between the spatial predictions of wolves and domestic dogs by combining the respective densities of each species (Figure 16), show that is inversely proportional, suggesting that a greater probability of wolf presence is related to a smaller probability of dog presence.

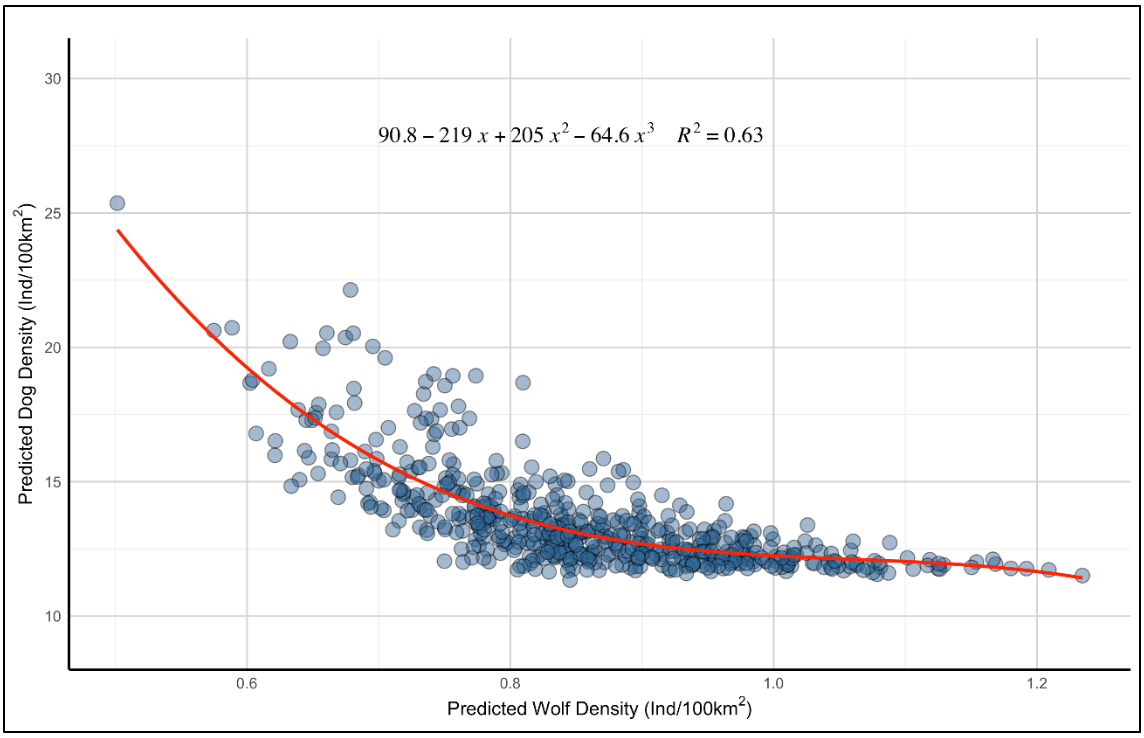


Figure 16 - Spatial predictions of wolf and dog densities in the study area located at south of Douro river.

Regarding to the data on dogs provided by municipal veterinarians, related to 2019 and early 2020, Aguiar da Beira and Moimenta da Beira are the municipalities with the highest number of dog registrations (n=200 and n=184, respectively) (Figure 16; Appendix VI). In relation to the estimated number of stray dogs, these municipalities have low numbers (n=10 and n=20, respectively) while Sátão has the highest number of reported stray dogs (n=109) as well as the highest number of abandoned animals collected by the municipality (n=75). Records of dog attacks on people or livestock were reported for several municipalities, including Moimenta da Beira (n=5), Aguiar da Beira (n=3), and Sátão (n=2) (Figure 17; Appendix VI).

Considering for each municipality the sum in the number of registered dogs, number of estimated stray dogs and number of collected abandoned dogs, we can estimate a population size of 227 dogs in Aguiar da Beira, 103 dogs in Mêda, 240 dogs in Moimenta da Beira, 266 dogs in Sátão and 81 dog registrations Vila Nova de Paiva. Based on these numbers in relation to the area covered by each municipality, the values obtain for dog density are: 8.2 dogs/km² in Aguiar da Beira, 0.4 dogs/km² in Mêda, 1.2 dogs/km² in Moimenta da Beira, 1.3 dogs/km² in Sátão and 0.5 dogs/km² in Vila Nova de Paiva, corresponding to an overall density of 11.6 dogs/km² in all study area.

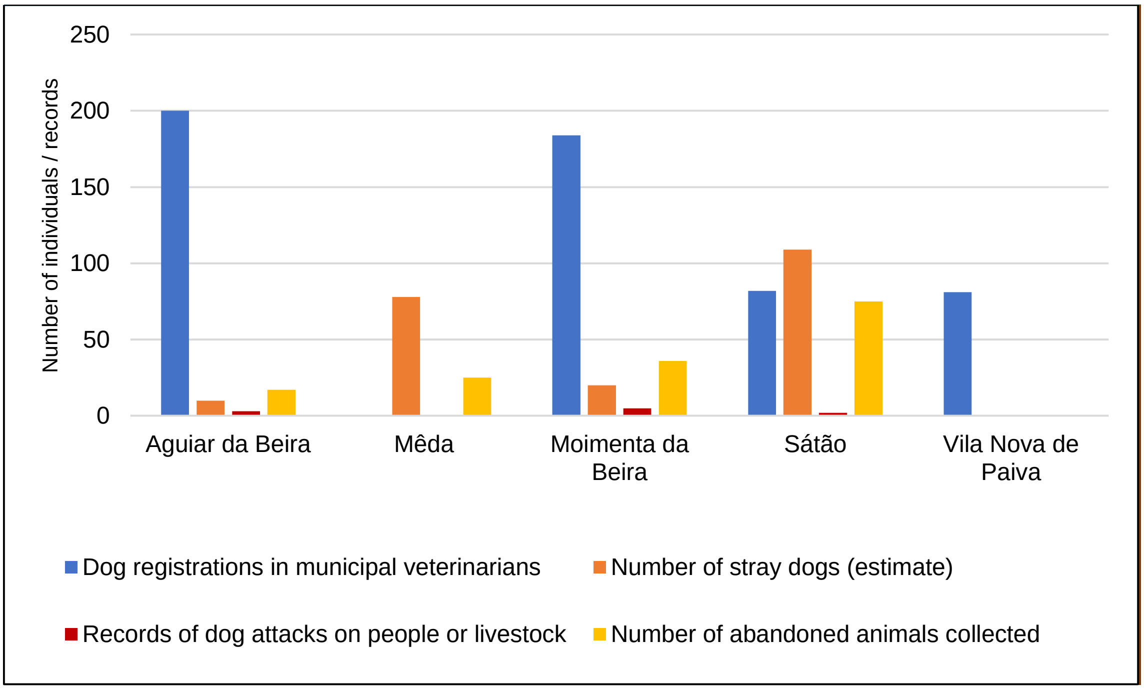


Figure 17 - Data provided by questionnaires to veterinarians, per municipality, including nº of dog registrations, estimated nº of stray dogs, nº of records of dog attacks on people or cattle and nº of abandoned dogs collected, during 2019 and early 2020.

Based on camera trapping data, it was possible to visually identify 43 different domestic dogs in the study area during 2018. Considering the territory assigned to each wolf pack, in the breeding areas of Lapa pack were detected 2 dogs (without collar identification or associated human presence); in Leomil area were detected 23 dogs (18 hunting dogs and the remaining 5 without associated human presence and collar identification); and in Trancoso area were detected 18 dogs (11 livestock guarding dogs, 1 pet dog associated to a human and 6 without human presence and identification). In total, 30% (n=13) of the detected dogs by camera trapping presented no evidences of being directly associated with humans or human activities, such as livestock husbandry and hunting.

We collected 15 samples from domestic dogs in the study area, including 1 sampled dog (found dead and with no identification) inside the territory attributed to Lapa pack, 10 sampled dogs inside the territory of Leomil pack (8 hunting dogs, 1 livestock guarding dog with goats and 1 livestock guarding dog with sheep), and 4 sampled dogs inside the territory of Trancoso pack (all livestock guarding dog with sheep). All dogs sampled in the study area matched with individuals visually identified through camera trapping in wolf breeding areas, but had no correspondence with the individuals genetically identified in dog scats between 2014 to 2018.

Activity patterns

The study on circadian activity of the Iberian wolf and the domestic dog was based on the analysis of 151 camera trap records (26 wolf records; 125 dog records), with a total sampling effort for camera-trapping of 230 night-traps between January 2018 and January 2019. From these records were obtained 69 independent records (19 wolf independent records; 50 dog independent records) The analysis of wolf and dog circadian activity based on camera-trapping reveal a difference in the activity levels and overlap between both species. Wolves show mainly a nocturnal activity pattern while domestic dogs shown a clear diurnal activity, with two peaks in late morning and late afternoon (Figure 18).

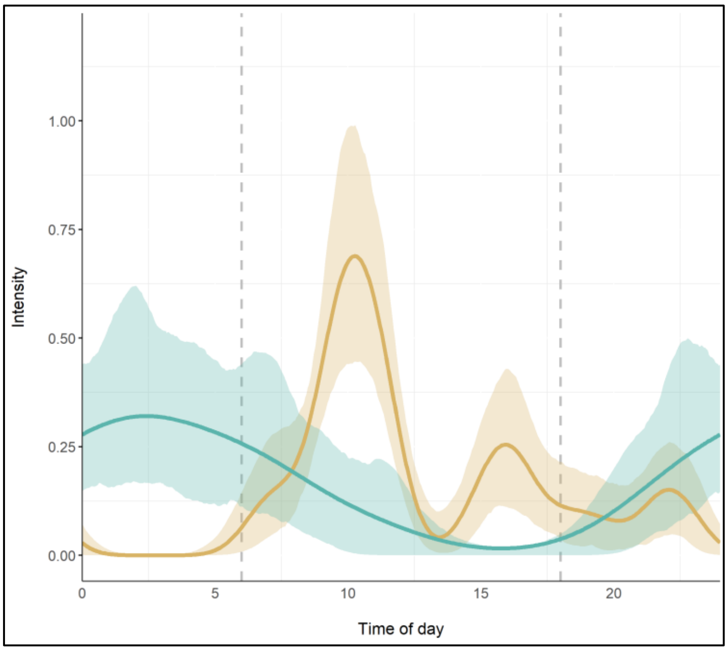


Figure 18 - Diel activity patterns of wolves (blue line) and domestic dogs (yellow line) in the study area at South of Douro river, based on camera-trapping in 2018. Vertical dashed lines represent sunset and sunrise times, respectively.

The number of active hours per day for wolves is 11.9 ± 2 (CI: 5.4 - 13.1), presenting a very large variability because there is a small amount of data. For dogs, the number of active hours per day is 5.5 ± 1 (CI: 3.9 – 7.9). The difference obtain between their activity levels is significant being 6.5 ± 2.2; *W*=7.87; *p*=0.01. There were also significant differences in the activity patterns of the two species (Δ1=0.34; *W*=12.03; *p*=0.002) with only 34% active simultaneously during a circadian cycle.

Diet composition

The analysis of diet composition was based on a total of 517 scats genetically confirmed as being from wolves (n = 247) and from domestic dogs (n = 270). The global results on diet composition reveal a high similarity in the feeding habits of both wolves and dogs considering all study area (Figure 19, Appendix VII), but some dietary differences considering the territory of each wolf pack (Figure 20, Appendix VIII), each sampling year (Figure 21, Appendix X) or each season (Figure 21, Appendix XII).

Overall, the prey classes identified for both wolves and dogs include ten species – six ungulates and four carnivores – and three taxonomic groups – Lagomorphs, Galliformes and Small Mammals, the later one only represented in dog diet (Figure 19, Appendix VII). In wolf diet, the consumption of wild ungulates is low (F.O. = 3.8%; Biomass = 5.3%), and represented only by one species (wild boar), while domestic ungulates (F.O. = 44.2%; Biomass = 58.5%) and Lagomorphs (F.O. = 30.4%; Biomass = 20.7%) comprise a much larger proportion of wolf diet. For wolves, the two basic resources are goats (F.O. = 28.3%; Biomass = 27.3%) and Lagomorphs, while regular resources were sheep and Galliformes, and supplementary and occasional resources, were wild boar (both adult and juvenile), horses, cattle, pig, badger, dog and cat (Figure 19, Appendix VII). Dogs diet show a similar pattern, with low consumption of wild ungulates (F.O. = 4.2%; Biomass = 5.1%) and high consumption of domestic ungulates (F.O. = 70.0%; Biomass = 78.5%), although there is a greater consumption of Galliformes (F.O. = 12.5%; Biomass = 8.0%) than Lagomorphs (F.O. = 8.3%; Biomass = 5.3%). Mesocarnivores are also present in dog diet with a small consumption (F.O. = 4.2%; Biomass = 2.9%), similar to what was found in wolf diet but with a different species composition (See Appendix VII). In contrary, small mammals, which are not present in the wolf diet, are only detected in dog diet although with a very low expression (F.O. = 0.4%; Biomass = 0.3%). For dogs, the only basic resource are goat and sheep, while regular resources are represented by Galliformes and Lagomorphs, the supplementary resources by wild boar, horse and badger, and finally, occasional resources by pig, fox and small mammals (See Appendix VII). In general, the biggest dietary differences between target species, is a higher consumption of domestic ungulates and a lower consumption of Lagomorphs by domestic dogs, compared to wolves. Based on these findings, the overlap of trophic niche between wolves and dogs is high (N.O. = 0.813), confirming the qualitative similarity on their diet. Regarding values of non-food items (e.g. plant and mineral material, insects, non-identified material), we also found similar patterns between wolf and dog scats, except for remains of garbage from human origin, which were only detected in nine dog scats (see Appendix I).

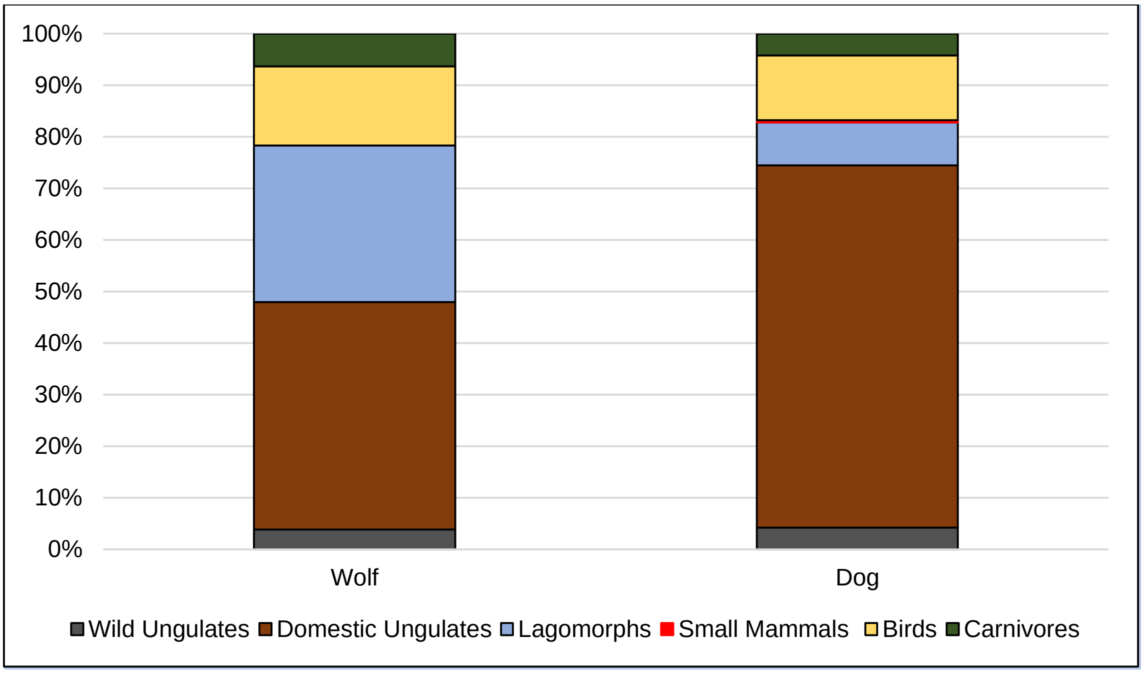


Figure 19 - Global results of wolf and dog diet in the study area located at South of Douro river. Values are expressed in F.O.

Regarding regional variation in the diet of wolf and dogs, considering each of the areas attributed to the three wolf packs, there are regional differences in diet composition, reflecting the local diversity and availability of prey species, and with target species also showing different feeding habitats in each area (Figure 20, Appendix VIII). In relation to wolf diet, the most consumed prey in the area of Lapa pack are Galliformes (F.O. = 43.9%), in Leomil pack are Lagomorphs (F.O. = 51.7%), while in Trancoso pack, are domestic ungulates, particularly goats (F.O. = 48.1%) (Figure 20, Appendix VIII). In relation to dog diet in the area of each of the three wolf packs, in Lapa the most consumed prey are goats (F.O. = 32.6%) and Galliformes (F.O. = 27.9%), in Leomil are goats (F.O. = 51.7%), while in Trancoso, are domestic ungulates, mainly sheep (F.O. = 46.1%) (Figure 20, Appendix VIII).

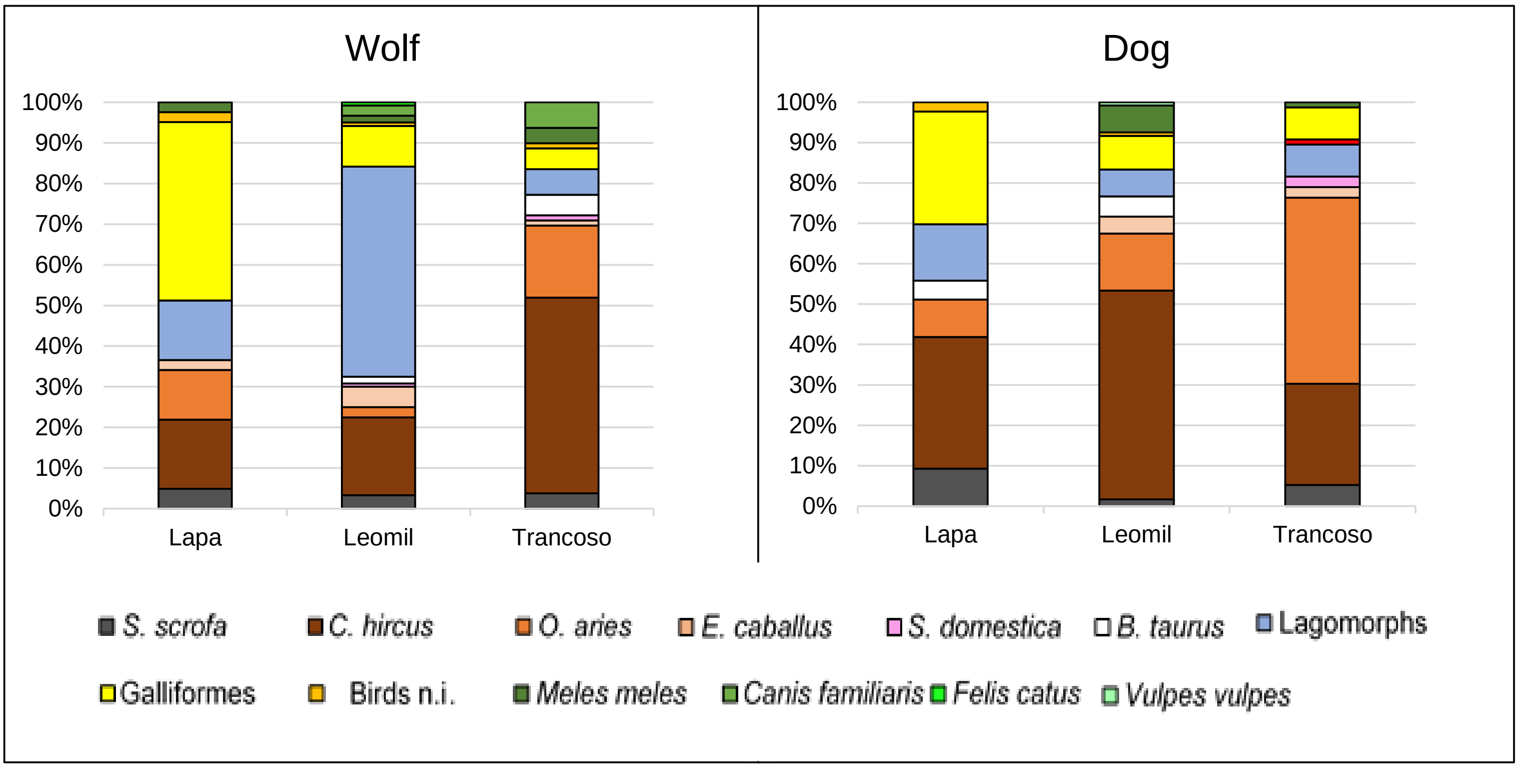


Figure 20 - Regional variation in the diet composition of wolves and dogs in South of Douro river, considering the area attributed to each wolf pack. Values are expressed in F.O.

Temporal variation in wolf and dog diet, considering each sampling year, also reveal differences in the diet composition between years and target species. In fact, both in wolf and dog diet, we found significant differences in the consumption of most prey species between sampling years, except for goat, sheep, Lagomorphs and Galliformes which have a similar consumption value in some of the sampling years (Appendix IX). For both target species, the main consumed prey across sampling years are domestic ungulates (F.O. > 30% for wolves; F.O. > 50% for dogs) with the exception of 2014 for wolves, in which Lagomorphs represent the most consumed prey (F.O. = 44.0%) (Figure 21, Appendix X). In relation to consumption of wild ungulates, this prey class represents constant and low values along all sampling years and between both target species, with F.O. ranging 3% to 7% and not being consumed in 2014 (for both target species) and 2017 (only for wolves). In contrary, there is some variation in the consumption of Galliformes and carnivores over the sampling years but with some concordance between target species (Figure 21, Appendix X).

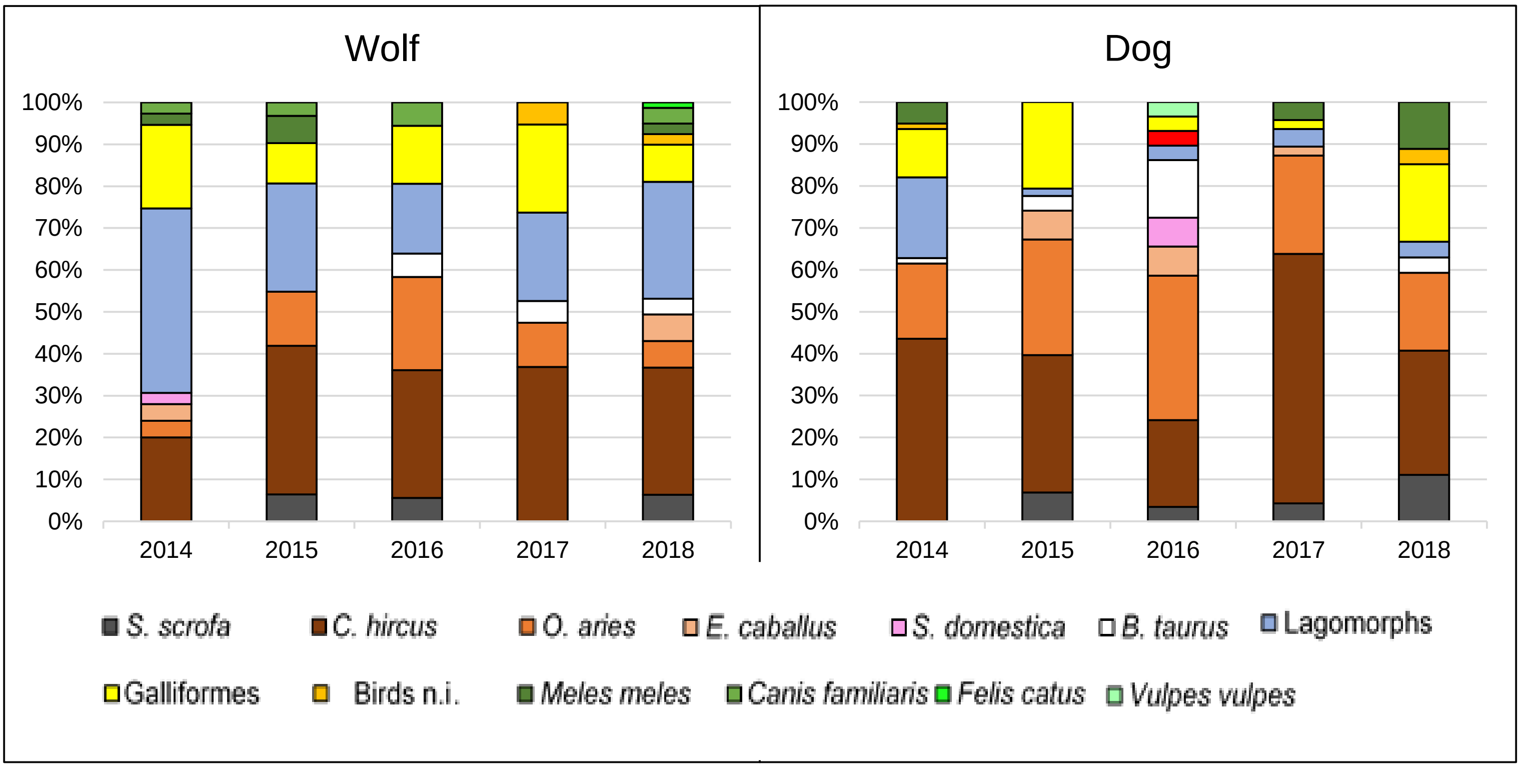


Figure 21 - Temporal variation in the diet composition of wolves and dogs in South of Douro river, considering each sampling year. Values are expressed in F.O.

Both wolves and dogs show significant differences in the consumption of most prey items between seasons, except for goat, lagomorphs, and Galliformes which have a similar consumption by wolves between some seasons (Appendix XI) and goat, sheep and Galliformes with similar consumption by dogs between Winter and Autumn and between Summer and Autumn (Appendix XI). Seasonal variation of wolf diet show that Lagomorphs are the main food item during Summer and Winter (F.O: = 28.8% and 42.6%, respectively) while goats are the most consumed prey in Spring and Autumn (F.O. = 33.3% and 39.5%, respectively) (Figure 22; Appendix XII). For dog diet, goats are the main food item during all seasons, with values of F.O. ranging from 27.9% in Autumn to 51.4% in Spring (Figure 22; Appendix XII). In addition, the consumption of Lagomorphs by dogs is higher during Summer and Autumn, reflecting a reverse pattern on goat consumption. For both target canids, all remain prey items are, in general, represented evenly across all seasons, with the exception of wild boars in wolf diet, which are more consumed during Autumn and Winter (Figure 22; Appendix XII).

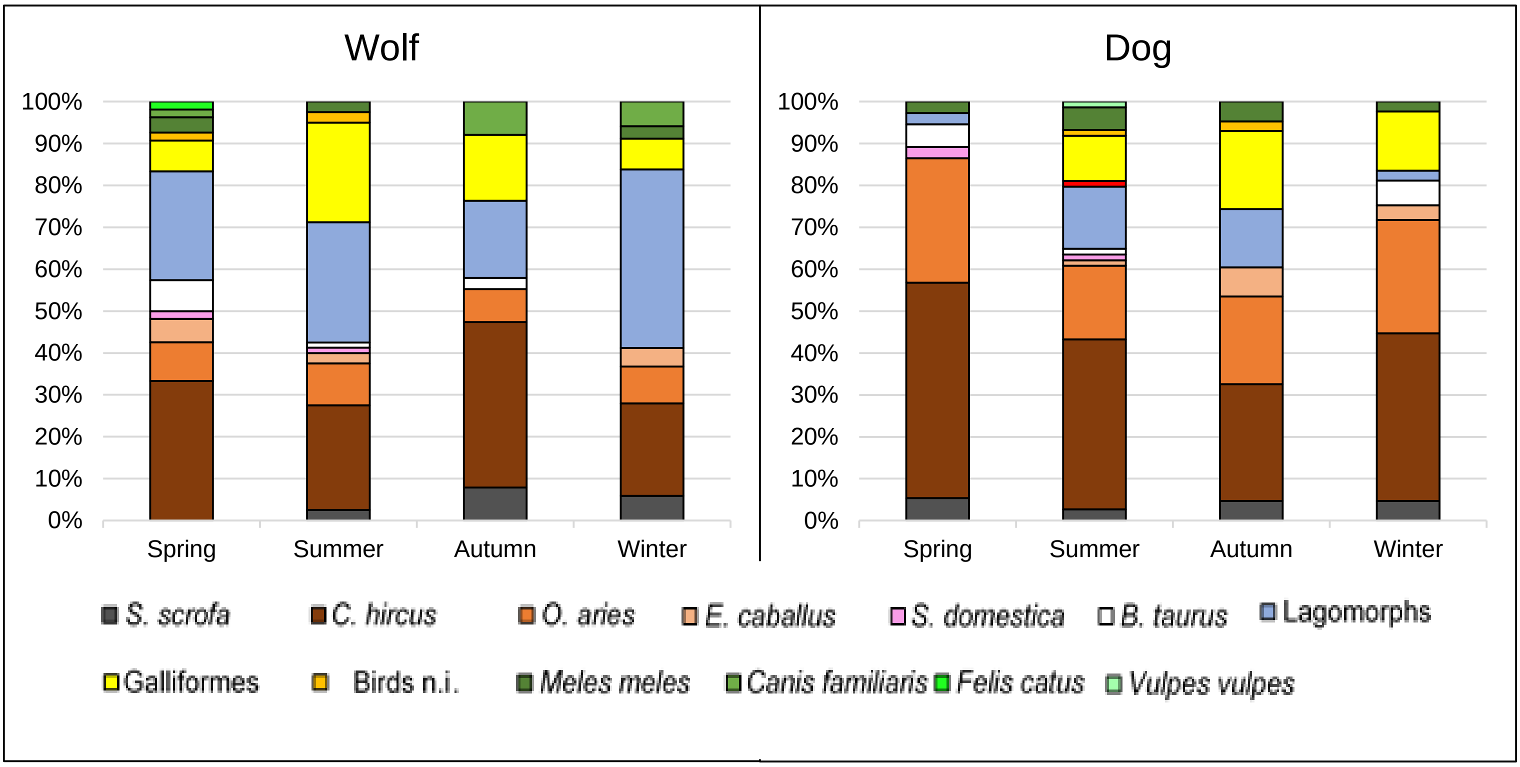


Figure 22 - Seasonal variation in the diet composition of wolves and dogs in South of Douro river, considering each seasonal period. Values are expressed in F.O.

The available data on domestic ungulates reported to be killed by wolves and declared to ICNF for compensation, considering the same area and period covered by diet analysis (2014 to 2017), revealed that sheep is the most affected prey by wolf predation (n=1176, 78% of all wolf attacks), followed by goats (n=191, 13%). However, an opposite pattern is evident in the dietary analysis of both wolves and dogs, where consumption of goats (F.O.≈ 60% in both canids) is much higher than sheep (F.O.= 24% and 34%, for wolves and dogs, respectively) (Figure 23; Appendix XIII). Regarding to other livestock species reported to be killed by wolves in the declared attacks, namely cattle (5%) and equines (2%), these are represented in a similar proportion than what was found in the diet of both wolves and dogs (F.O. Cattle = 5% for both canids; F.O. Equines = 4%, for both canids). Finally, a few number of domestic dogs were also reported to be killed by wolves in the attacks declared to ICNF (n = 4, 0.3%), which is accordingly to the dietary results where domestic dogs are also represented as a prey item in wolf diet (n=8; F.O.=7%) although absent in dog diet (Figure 23; Appendix XIII).

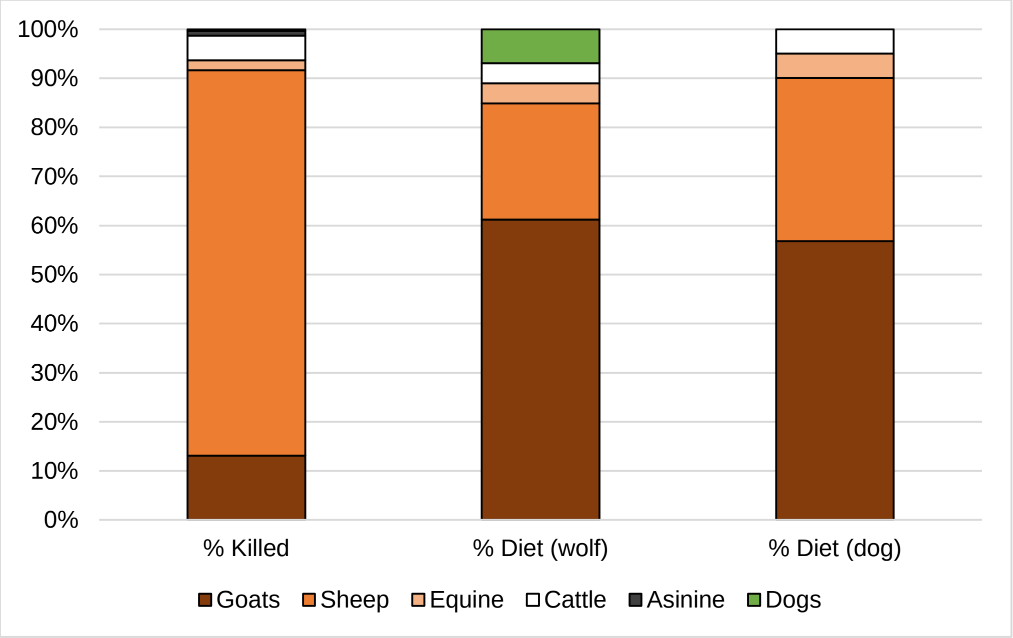


Figure 23 - Proportion of each livestock species considering the number of animals killed by wolves declared to ICNF (% Killed) and the value of F.O, in scats of wolves and dogs (% Diet), in all study area at South of Douro river, between 2014 and 2017

5. Discussion

This study provided valuable insights on spatial, temporal and trophic niche of Iberian wolves and sympatric domestic dogs, in a human dominated landscape, by assessing abundance, population estimates, spatial interactions, circadian activity and diet composition of these two close-related canids. This is the first study on wolf-dog interactions in Portugal and one of the few at a European level. Therefore, it addresses a topic with management relevance and worldwide implications, as free-ranging domestic dogs are considered a threat not only for wolf conservation but for many other wildlife species. With relevance at a regional level, this study also provides updated ecological knowledge on the endangered wolf population located in Portugal at south of Douro river.

Population estimates and spatial niche

The estimated population size of wolves and domestic dogs in all study area and per each pack territory proved that free-ranging dogs are more abundant than wolves, corroborating our first study hypothesis and accordingly to other studies (Boitani, 1983; Wierzbowska *et al.,* 2016). In the case of wolves, we found more males than females, with a slight decrease over the years based on the model average. This pattern is consistent with the results from continuous population monitoring of these packs, which report a decrease in breeding rates and pack size along the last decades (Serronha *et al.,* 2019). The abundance of dogs also has more tendency to decrease based on the model average and also, being notable the presence of more males than females. However, it should be considered that, although wolf population estimates must be accurate, the estimated population of dogs in our study area corresponds to a fraction that occur inside wolf range and was detected based in a sampling targeting scats morphologically attributed to wolves. The population estimates and density obtained through the data from municipalities, only represents a small portion of dogs when compared with the values of the models that represent all study area. Even so, the dogs' largest densities are included in several municipalities that belong in the area where wolves are present or breed regularly (Lapa and Leomil). It is not possible to generalize these values due to their local characteristics, so further studies on this topic are necessary.

The 15 samples collected from domestic dogs in the study area, can describe briefly the characteristics of a few dogs present in this area, being mostly livestock guarding dogs and hunting dogs that may be associated to a stronger interaction with wildlife

(Lescureux & Linnell, 2014). They are present in wolf breeding areas, but since they had no correspondence with the individuals genetically identified in dog scats, further studies will be needed to ascertain their connection with wolves.

Contrary to the literature, our prediction of higher dog density close to humans was not confirmed (Hughes & Macdonald, 2013) contradicting part of our second study hypothesis. However, this observed pattern can be due to the fact that dog scats near human buildings are not collected as they are discarded in the wolf-orientated sampling that was conducted (Serronha *et al.,* 2019). On the other way, and in compliance with the collecting procedure followed by Serronha et al., (2018), the relation between dog density and the distance to wolf breeding areas, shows that all dog scats are within the theoretical centers of activity and within the sampled transects with a constant value representation, because they are not discarded by the sampling team. In fact, dogs are usually accompanied by people, such as livestock guarding dogs and hunting dogs, and roam all study area confirming the additional part of our second study hypothesis (Boitani & Ciucci, 1995).

We must emphasise that all dog scats have been collected by the detection teams embodied in wolf-related projects because they have a wolf-like appearance and morphology but they were then genetically proven to be dog, and therefore should be considered a limitation for the performance of this work. In other words, sampling is limited to certain conditions and scats of the two species are not collected for the same purpose because the team only collects samples that might be wolf. Therefore, sampling bias can be considered a limitation leading to a systematic over- or under-estimation of the abundance and density of dog population. In addition, there is also a bias of sampling of dogs because it has never been sampled around large cities but only on routes where there is a greater indication of the presence of wolf. These two factors influence the results because the collection is smaller and underrepresents the dog population.

The density of dogs decreases along with an increase in wolf density. This relationship is a correlational interpretation of the outputs of the two models made independently. In fact, there is a spatial interaction between the two species in wolf's territory (Boitani & Ciucci, 1995). But, it is also possible to detect that dogs are more dispersed and present in the wolf territory (Boitani & Ciucci, 1995; Wierzbowska *et al.,* 2016). In fact, we observe a spatial segregation of dogs in wolf breeding sites since dogs' density is present not only at the limits with the highest presence of wolf density, for the three packs, or in places whose densities are lower or non-existent (Vanak *et al.,* 2014). These two findings are corroborated by our third study hypothesis. This could be explained by the possibility of the dominant character of the wolves, that could fright the dogs and push them to the limits of their territory, opposed to Atickem et al., (2010) findings.

The areas of higher wolf density in our study area, which perfectly reflect the territories for the three studied packs, are related to higher altitudes, as these provide better conditions of refuge and tranquillity towards human activity, as described extensively in other studies (Mech 1989; Theuerkauf *et al.,* 2003b; Capitani *et al.,* 2006; Habib & Kumar, 2007; Person & Russell, 2009; Ahmadi *et al.,* 2014; Iliopoulos *et al.,* 2014; Sazatornil *et al.,* 2016) corroborated by our fourth study hypothesis. But, the variation in wolf density as a function of distance to human build up can be interpreted by the fact that human constructions are scattered in the study area, and therefore considered this variable as moderately informative in relation to the spatial pattern of wolf density. In addition, based on our results, wolves seem more associated with human constructions than dogs. This may probably be related to the fact that many of these buildings being uninhabited and in ruins, and situated in areas with low human presence. This data is encouraging since it may be assigned of the wolves' behavioural plasticity (Eggermann *et al.,* 2011).

Concerning the detection probability, data showed a decline in the probability of detection over the years and starting to rise from 2017, reaching the highest values in 2018. This decrease may be related to various factors. One of the hypotheses is that experienced observers could be more tired or uninterested and new observers may perform better (Soller *et al.,* 2020). Boredom and fatigue have been known to influence observer performance in wildlife surveys (Norton-Griffiths, 1976). The effect of individual variation in fatigue, boredom, visual acuity, and experience on sample success are complicated to evaluate (Neff, 1968) but these factors have the potential to bias results especially when implementing the same individual observers over time (Soller *et al.,* 2020). In the case of our study, there was a change in the detection team in 2017 and after this change is reached the highest value of probability of detection. Therefore, the physical and psychological factors should always be considered.

Also, our data indicated that wolf females are more detectable than males, contrary to dogs. Wolf females mark with greater intensity in the central areas of the territory while the males mark more extensively in the territory. That is, the females mark more within the transects unlike the males. This may be due to the fact that females are more on breeding sites and males have a more territorial pattern (Sazatornil *et al.,* 2016). For dogs, there is no difference in detection probability between males and females both behave the same way. Further research on this topic would be relevant.

Temporal niche of wolves and dogs

According to previous studies on wolf activity patterns, this large carnivore is more active during crepuscular periods, as their activity peak at dawn and dusk, but also includes some activity during daytime (Ciucci *et al.,* 1997; Theuerkauf *et al.,* 2003a, c; Rio-Maior *et al.,* 2018). However, our results that were focused on wolf breeding sites, show a mostly nocturnal activity, with no evident peaks at crepuscular periods. This may be explained by a lower wolf activity during the day, since wolves require shelter from the sun when travelling, especially in open areas (Theuerkauf *et al.,* 2003a). Besides, there are other relevant factors that can influence the circadian activity of wolves, such as human activity and presence (Vilà, 1995; Ciucci *et al.,* 1997; Kusak *et al.,* 2005). By co-inhabiting with humans, wolves at south Douro river may adjust their activity patterns in order to temporally segregate from humans, thereby facilitating their foraging movements (Theuerkauf *et al.,* 2003c). Furthermore, studies carried out at south of Douro river with wolves monitored by telemetry revealed that wolves have lower activity during the morning and afternoon, being mostly nocturnal (Grilo *et al.,* 2002b), which coincides with our results obtained by camera trapping. Other studies resorting to wolf GPS telemetry in the region of Montalegre/Xinzo de Limia (whose habitat is very humanized and similar to south of Douro region) also revealed a greater activity during the night and twilight period and less activity in the daytime (Álvares, 2011). In contrary, the circadian pattern of GPS collared wolves in areas with lower human disturbance, such as Montesinho Natural Park (Bragança), revealed that wolves despite a bimodal crepuscular activity, are also active during all hours of the day, although with less activity between 10am and 5pm (Moreira, 1992; Pimenta, 1998). A final alternative explanation for our results may be related to the presence of dogs in the same area as found in the current study.

Concerning dogs, our results from camera-trapping inside wolf core areas at south Douro river showed an almost strictly diurnal activity on free-ranging dogs, more active in the morning and early afternoon, as found by Boitani (1983). Concerning the stray dogs, the same author showed that they can be active both during daytime and night, although adjusting their activity pattern near human presence (Boitani, 1983). In relation to free-ranging domestic dogs, as they are dependent to owners for food and shelter, the activity patterns are mostly diurnal (Boitani, 1983), which is in accordance to our results and our study hypothesis. In fact, wolves and dogs in our study area seem to segregate temporally, being wolves mostly nocturnal and dogs' diurnal.

Trophic niche of wolves and dogs

The diet composition of the two canids showed a high similarity between the consumed prey in south of Douro region, being mainly composed by domestic ungulates. The trophic niche overlap between wolves and dogs in our study area, revealed that both canids feed on the same resources thus proving our first hypothesis regarding to this topic (Wierzbowska *et al.,* 2016).

Livestock predation by wolves is considered a worldwide concern and domestic ungulates can become a primary prey for wolves, mainly dependent of the type of husbandry practices (Barja, 2009; Iliopoulos *et al*., 2009; Vos, 2000; Torres *et al.*, 2015a). Most of this studies document wolf consumption on a few livestock species under extensive husbandry, such as goats, sheep and cattle (Cuesta *et al*., 1991; Lançós, 1999; Vos, 2000; Torres *et al*., 2015a). However, our study presents a much wider range of domestic animals consumed by wolves in south of Douro region, including other domestic animals usually confined in intensive production farms, such as horses, rabbits, poultry and domestic pigs, similarly to what has been previously reported for some of the studied wolf packs (Casimiro, 2017). In general, there are few studies of the ecology of dogs (Vanak & Gompper, 2009; Young *et al.,* 2011; Wierzbowska *et al.,* 2016). Regarding to domestic dog predation, the consume of the same domestic preys proves a connexion between the two carnivores. The results obtained regarding dogs' diet are the first performed in this area of study. These results need further research to confirm a persistent pattern.

In south of Douro region, is applied an extensive husbandry for sheep and goats, a semi-confined system for cattle, horses and donkeys and an intensive production in closed farms for domestic pig, rabbits and poultry (Torres *et al.,* 2015a; Pimenta *et al.,* 2017). This variety in husbandry practices involves different levels of prey availability both for wolf and dog. Therefore, most of the domestic animals that were identified in wolf diet, imply distinct feeding strategies based on either a predatory behaviour (e.g. sheep, goats and cattle) or a scavenging behaviour for the remain domestic species that are confined (e.g. poultry, lagomorphs and pigs). Most of these animal remains are deposited outside these facilities, being more accessible to wolves and dogs. Regarding to dog diet, they also fed on animal remains from intensive farms and, in addition, from remains of animals killed by wolves, revealing scavenging behaviour (Butler & du Toit, 2002; Vanak *et al.,* 2014).

These evidences are also supported by our comparisons between dietary results and the reported attacks to ICNF in the study area, suggesting that domestic pigs, poultry and, particularly, domestic rabbits may be consumed on dumpsites with carcasses from intensive farms. Probably, the big difference between goats and sheep in the dietary results, and the reported attacks to ICNF, may suggest that the consumption of goats is related with necrophagic behaviour by the two canids and the sheep consumption of dogs could be related to the remains of sheep killed by wolves corroborating our second study hypothesis.

Regarding to carnivore prey, they are included in our results as be part of wolf diet as a supplementary or occasional resource. An increased number of different carnivore species in wolf diet could signalize loss of biodiversity and instable habitats (Brito, 2017). That fact was confirmed by the declared loss to ICNF of four dogs, and this prey class was also found in the analysis of wolf scats. The highly anthropogenic changes of this region, could justify their consumption and consequently pushes wolves to get closer to humanized areas. The presence of carnivores in dogs' diet may be related to hunting activities and is also a supplementary or occasional resource.

These results are similar to the findings from Casimiro (2017) for Leomil pack. In fact, the consumption of lagomorphs in the study area appears to be essential to the survival of wolves being quite notorious in Leomil's pack. In previous studies (Quaresma, 2002; Sobral, 2006; Pinto, 2008), this resource was not so representative in wolf diet (maximum value of 13% F.O.) contrary to the study of Casimiro (2017) where was represented by 60% of F.O. in Leomil pack. In our study, it reaches the F.O. value of 30.4% (being consumed mostly by the pack of Leomil) regarding to the three packs and is considered a basic resource. The availability of this food resource depends on the accessibility to wolves of intensive farms and respective dump sites where carcasses are left (Alexandre *et al.,* 2000). The difference along sampling years on the consumption pattern on domestic ungulates and lagomorphs by wolves and dogs in south Douro river, may be caused by changes in husbandry practices, implemented measures to prevent wolf attacks and the exodus of the rural population (Vos, 2000; Torres *et al.,* 2015a). The numbers of domestic ungulates in extensive grazing systems are declining (Vos, 2000) and this factor might represent a threat to wolves, considering their dependence on human related-food resources often scattered, such as carcasses on dumpsites. Regarding to the consumption between seasons, Winter presents the lowest values of domestic prey in the detected prey in wolf scats (35.3% F.O.). On the other hand, this is the season with the highest consumption of lagomorphs (42.6% F.O.), which could be connected with variations in the activity of wolves near farms, where they may search more often for food, by scavenging in animals remains left in dumps (Llaneza & López-Bao, 2015). In dogs' diet, goats are the main food item during all seasons, with values of F.O. ranging from 27.9% in Autumn to 51.4% in Spring. This may be related again, with their necrophagic pattern but further research is needed to confirm this assumption.

The fact that these two canids share the same food resources may lead to a greater factor of competition between them (Wierzbowska *et al.,* 2016). It will be necessary to infer further ecological studies framing these two species.

5.1. Conclusions and management implications

This work aimed to investigate the ecological interactions between wolves and dogs in a specific region of Portugal. Our findings suggested an overlap between wolves and dogs at the trophic and spatial niche but not at the temporal level thus supporting the different hypotheses addressed regarding the ecological interactions between these two species in a human-dominated landscape.

In many European countries is sanctioned to abandon dogs, however most countries have slight knowledge on the size and trends of their stray/feral dog populations (Tasker, 2007). In our study, only six municipalities provided us information regarding to stray dogs and only referring to one-year period. Furthermore, this information was based on guess estimates, therefore with a high level of uncertainty about the effective size of these populations. According to recent news on social media (Jornal Público, 2020a, b; TSF, 2020), a sharping increase in feral dogs in Portugal persists because the collection centres and the associations are unable to gather more animals and the municipalities seem powerless to deal with this problem.

In the Action Plan for Wolf Conservation in Portugal, the predation that dogs exert on domestic animals is a negative factor for the conservation of the wolf, particularly in Portugal (Álvares *et al.,* 2015). Widespread scepticism about the possibility of dogs attacking livestock, and the costs associated with using techniques that allow determining with reliability the predator responsible for the attack, cause the damage to be normally attributed to the wolf (Álvares *et al.,* 2015). This supports and escalating of the conflict between rural communities and the wolf, making it difficult to create the necessary conditions for the conservation of this predator (Álvares *et al.,* 2015). In our work, we only found 9 attacks of dogs to livestock, a number that is considered low in comparison with number of wolf attacks.

As previously recommended by researchers (Álvares *et al.,* 2015) and in line with our results, only effective legislation enforcement regarding to the possession and circulation of dogs, the education and information of the public, monetary indemnity by attacks of stray/feral dogs to livestock, as well as the control of stray or wandering dogs, will effectively solve this problem.

Regarding to the management projects for conservation of wolf in Portugal, they should be re-evaluated. An efficient management of conflicts between wolf and husbandry systems constitutes a key element of a sustainable strategy for the conservation of this species. Therefore, the growth of wild prey numbers, develop efficient livestock husbandry practices and eliminate monetary incentives that reward poor management practices are priorities (Boitani, 2000; Mech *et al.,* 2000; Treves *et al.,* 2004; Bradley & Pletscher, 2005; Dondina *et al.,* 2014).

The methodological approach applied in this study should be extended to other regions that have a sympatric presence of these two canids under different ecological conditions. This will offer additional knowledge on wolf-dog interactions thus providing greater awareness to the ecological impact of free-ranging and feral dogs. Furthermore, our data enable a better understanding on the resource competition between these two canids as well as the spatial, temporal and behavioural strategies that both wolves and dogs use to occur in sympatry. This knowledge has strong implications to support a better management for the conservation of the Iberian wolf and the improvement of human-wolf relationships, particularly in the endangered wolf subpopulation located at south of Douro river. It is essential to alert the community in general, but also the entities responsible for the current situation with free-ranging dogs about the implications of the existence of stray/feral dogs, and their strong impacts not only ecologically and economically, but also in public health.

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TSF, 2020: https://www.tsf.pt/portugal/sociedade/ha-um-descontrolo-completo-dos-caes-e-gatos-esta-tudo-superlotado-11791091.html Assess at 06/10/2020

Appendix

Appendix I - Number of occurrences and respective percentage of non-food material items, identified macroscopically in wolf and dog scats collected in the study area at south of Douro river. These items consist in vestigial hairs from the target species, non-identified material, bones, purgative plants, mineral materials, plant leaves, insects, and garbage.

Wolf		
Unanalysed constituents	**N**	**%**
Hairs	58	23.5%
Brachypodium phoenicoides	78	31.6%
Plants	71	28.7%
Mineral materials	65	26.3%
Bone materials	143	57.9%
Insects	6	2.4%
Garbage	0	0.0%
Not identified material	1	0.4%
Animal not identified	5	2.0%

Dog		
Unanalysed constituents	**N**	**%**
Hairs	54	20.0%
Brachypodium phoenicoides	67	24.8%
Plants	110	40.7%
Mineral materials	58	21.5%
Bone materials	166	61.5%
Insects	5	1.9%
Garbage	9	3.3%
Not identified material	7	2.6%
Animal not identified	12	4.4%

Appendix II - Average weights of each prey item detected in wolf and dog diet in the study area at south of Douro river, used to estimate Consumed Biomass.

Prey class		Mean weight (Kg)	References
Wild ungulates	*Capreolus capreolus*	24.0	Pimenta (1998)
	Sus scrofa	22.0 (juvenile) 67.0 (adult)	Llaneza *et al.,* (1996)
Domestic ungulates	*Equus caballus*	200.0	Álvares (1995)
	Equus asinus	180.0	Álvares (1995)
	Bos taurus	300.0	ICN (1997)
	Sus domestica	135.0	Bastos (2001)
	Capra hircus	25.0	DRAEDM (1993)
	Ovis aries	20.0	DRAEDM (1993)
Carnivores	*Canis familiaris*	15.0	Moreira (1992)
	Felis sp.[1]	3.9	Moreira (1992); López-Martín *et al.,* (2011)
	Meles meles	7.3	Revilla *et al.,* (2011)
	Vulpes vulpes	6.7	(Hattingh, 1956)
Lagomorphs		1.5	Moreira (1992)
Small Mammals		0.02	Macdonald & Barret (1993)
Galliformes		1.85	Quaresma (2002)

[1] *Considered as a mean value between the weights of the wildcat and the domestic cat.*

Appendix III - Model results calculated to evaluate the density (D~), baseline detection (p~), the spatial scale of detection (sig~) and cost function (asu~) for wolves and domestic dog in the study area at south of Douro river, between 2014 and 2018, considering different covariates (zRBuildUp - distance to the nearest human build up; zdCAct - distance to wolf breeding sites; zAlt - mean altitude) and combinations of the effects (~1 for no effects; ~session for year-specific effects; ~Tr for assessing potential trends; ~sex for sex effects).

Iberian wolf models	**LogL**	**K**	**AIC**	**dAIC**	**weight**	**CumWt**
D(~zRBuildUp) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.36	12.00	1 622.72	0.00	0.21	0.21
D(~zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.42	12.00	1 622.83	0.11	0.20	0.41
D(~Tr) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.43	12.00	1 622.85	0.14	0.20	0.61
D(~zRBuildUp + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.97	13.00	1 623.94	1.22	0.11	0.72
D(~Tr + zRBuildUp) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.13	13.00	1 624.26	1.54	0.10	0.82
D(~Tr + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.19	13.00	1 624.38	1.66	0.09	0.91
D(~Tr + zRBuildUp + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.75	14.00	1 625.50	2.78	0.05	0.96
D(~session) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.17	15.00	1 628.34	5.62	0.01	0.97
D(~session + zRBuildUp) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.89	16.00	1 629.77	7.05	0.01	0.98
D(~session + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.92	16.00	1 629.85	7.13	0.01	0.99
D(~Tr + session) p(~session + sex + zdCAct) sig(~sex) asu(~1)	799.17	16.00	1 630.34	7.62	0.00	0.99
D(~session + zRBuildUp + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.50	17.00	1 631.00	8.28	0.00	0.99
D(~session + Tr + zRBuildUp) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.89	17.00	1 631.77	9.05	0.00	1.00
D(~session + Tr + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.92	17.00	1 631.85	9.13	0.00	1.00
D(~session + Tr + zRBuildUp + zAlt) p(~session + sex + zdCAct) sig(~sex) asu(~1)	798.50	18.00	1 633.00	10.28	0.00	1.00
Domestic dog models						
D(~session + zRBuildUp) p(~session) sig(~1) asu(~1)	814.00	13.00	1 654.01	0.00	0.30	0.30
D(~session) p(~session) sig(~1) asu(~1)	815.27	12.00	1 654.53	0.52	0.23	0.53
D(~session + zRBuildUp + zdCAct) p(~session) sig(~1) asu(~1)	814.00	14.00	1 656.01	2.00	0.11	0.64
D(~session + Tr + zRBuildUp) p(~session) sig(~1) asu(~1)	814.00	14.00	1 656.01	2.00	0.11	0.75
D(~session + zdCAct) p(~session) sig(~1) asu(~1)	815.22	13.00	1 656.44	2.43	0.09	0.83
D(~Tr + session) p(~session) sig(~1) asu(~1)	815.27	13.00	1 656.53	2.52	0.08	0.92
D(~session + Tr + zRBuildUp + zdCAct) p(~session) sig(~1) asu(~1)	814.00	15.00	1 658.01	4.00	0.04	0.96
D(~session + Tr + zdCAct) p(~session) sig(~1) asu(~1)	815.22	14.00	1 658.44	4.43	0.03	0.99
D(~Tr + zRBuildUp) p(~session) sig(~1) asu(~1)	821.64	10.00	1 663.28	9.28	0.00	0.99
D(~Tr) p(~session) sig(~1) asu(~1)	823.12	9.00	1 664.25	10.24	0.00	1.00
D(~zRBuildUp) p(~session) sig(~1) asu(~1)	823.47	9.00	1 664.95	10.94	0.00	1.00
D(~Tr + zRBuildUp + zdCAct) p(~session) sig(~1) asu(~1)	821.64	11.00	1 665.28	11.28	0.00	1.00
D(~Tr + zdCAct) p(~session) sig(~1) asu(~1)	823.08	10.00	1 666.16	12.16	0.00	1.00
D(~zRBuildUp + zdCAct) p(~session) sig(~1) asu(~1)	823.47	10.00	1 666.94	12.94	0.00	1.00
D(~zdCAct) p(~session) sig(~1) asu(~1)	824.84	9.00	1 667.69	13.68	0.00	1.00

Appendix IV - Estimate abundance of the Iberian wolf and the domestic dog in South of Douro region between 2014 till 2018, considering different covariates (Tr -Trend; zRBuildUp - Build up; zAlt - Altitude; zdCAct - Activity center).

	Estimate ± se	**Lower**	**Upper**	**session**	**Tr**	**zRBuildUp**	**zAlt**	**sex**
Wolf	7.56 ± 2.24	4.23	13.51	2014 - 2018	1	0	0	Female
	11.92 ± 2.44	7.98	17.81		1	0	0	Male

	Estimate ± se	**Lower**	**Upper**	**session**	**Tr**	**zRBuildUp**	**zdCAct**	**sex**
Dog	131.94 ± 38.49	74.48	233.73	2014	1	0	0	Female
	215.61 ± 60.53	124.36	373.79	2014	1	0	0	Male
	37.52 ± 12.87	19.16	73.48	2015	2	0	0	Female
	61.31 ± 20.19	32.15	116.93	2015	2	0	0	Male
	0.00 ± 0.00	0.00	0.00	2016	3	0	0	Female
	0.00 ± 0.00	0.00	0.00	2016	3	0	0	Male
	127.43 ± 59.78	50.81	319.61	2017	4	0	0	Female
	208.24 ± 95.65	84.64	512.35	2017	4	0	0	Male
	48.33 ± 15.75	25.51	91.54	2018	5	0	0	Female
	78.97 ± 24.61	42.88	145.44	2018	5	0	0	Male

Appendix V - Untransformed model-averaged coefficients of the Iberian wolf and the domestic dog in South of Douro region between 2014 till 2018 the density (D~), baseline detection (p~), the spatial scale of detection (sig~) and cost function (asu~) for wolves and domestic dog in the study area at south of Douro river, between 2014 and 2018, considering different covariates (zRBuildUp - distance to the nearest human build up; zdCAct - distance to wolf breeding sites; zAlt - mean altitude) and combinations of the effects (~1 for no effects; ~session for year-specific effects; ~Tr for assessing potential trends; ~sex for sex effects).

Wolf				
Parameter	**Estimate ± Estimate Standard**	**Error Estimate***	**Standard Error***	**Relative valiable importance**
d.beta.session2	-0.21 ± 21.18	-0.01	0.82	0.04
d.beta.session3	-0.20 ± 42.06	-0.01	1.63	0.04
d.beta.session4	-0.39 ± 62.92	-0.01	2.44	0.04
d.beta.session5	-0.13 ± 83.73	-0.005	3.23	0.04
d0.(Intercept)	-3.24 ± 1.07	-3.24	1.07	1.00
p0.(Intercept)	-2.86 ± 0.38	-2.86	0.38	1.00
p0.male	-0.63 ± 0.35	-0.63	0.35	1.00
p0.session2	-0.57 ± 0.45	-0.57	0.45	1.00
p0.session3	-0.87± 0.46	-0.87	0.46	1.00
p0.session4	-0.70 ± 0.47	-0.70	0.47	1.00
p0.session5	0.29 ± 0.33	0.29	0.33	1.00
psi.constant	0.47 ± 0.35	0.47	0.35	1.00
sig.(Intercept)	7.98 ± 0.11	7.98	0.11	1.00
sig.sexmale	0.51 ± 0.13	0.51	0.13	1.00
t.beta.zdCAct	-1.28 ± 0.13	-1.28	0.13	1.00
d.beta.Tr	-0.07 ± 1.89	-0.03	0.87	0.45
d.beta.zRBuildUp	-0.16 ± 0.22	-0.08	0.16	0.46
d.beta.zAlt	0.13 ± 0.18	0.06	0.12	0.47

Dog				
Parameter	**Estimate ± Estimate Standard**	**Error Estimate***	**Standard Error***	**Relative valiable importance**
d.beta.session2	-1.44 ± 0.82	-1.43	0.82	0.99
d.beta.session3	12.66 ± 2.75	12.55	2.86	0.99
d.beta.session4	-0.54 ± 2.04	-0.54	2.03	0.99
d.beta.session5	-1.70 ± 2.59	-1.69	2.58	0.99
d0.(Intercept)	-0.58 ± 0.73	-0.58	0.73	1
p0.(Intercept)	-2.75 ± 0.25	-2.75	0.25	1
p0.session2	0.62 ± 0.43	0.62	0.43	1
p0.session3	-14.60 ± 2.19	-14.60	2.19	1
p0.session4	-0.83 ± 0.52	-0.83	0.52	1
p0.session5	0.51 ± 0.41	0.51	0.41	1
psi.constant	0.49 ± 0.20	0.49	0.20	1
sig.(Intercept)	6.98 ± 0.08	6.98	0.08	1
d.beta.Tr	0.63 ± 1.76	0.17	0.63	0.27
d.beta.zRBuildUp	0.11 ± 0.07	0.06	0.08	0.56
d.beta.zdCAct	-0.02 ± 0.11	-0.004	0.03	0.27

Appendix VI - Data provided by questionnaires to municipal veterinarians, related to number of dog registrations, estimated number of stray dogs, number of recorded dog attacks to people or cattle and number of abandoned animals collected, for each municipality in the study area at south of Douro river during 2019 and early 2020.

Municipality	Number of domestic dogs registered by municipal veterinarians [1]	Number of stray dogs (guess estimate)	Number of recorded attacks to cattle or people	Number of abandoned dogs collected by the municipality
Aguiar da Beira	200	10	3 attacks to livestock	17
Castro Daire	No information obtained	No information obtained	No information obtained	No information obtained
Mêda	No record	78	No record	25
Moimenta da Beira	184	20	5 attacks to sheep and goat.	36
Penedono	No information obtained	No information obtained	No information obtained	No information obtained
Sátão	82	109	1 attack to people 1 attack to cattle	75
Tarouca	No information obtained	No information obtained	No information obtained	No information obtained
Trancoso	No information obtained	No information obtained	No information obtained	No information obtained
Vila Nova de Paiva	81	No record	Unaware of any case in the last 2 years	The municipality doesn´t have animal collection centre (dog kennel)
Sernancelhe	No information obtained	No information obtained	No information obtained	No information obtained

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[1] Based on the Rabies Vaccination campaign and electronic identification. It should be noted that the vaccine is triennial, and all animals are not vaccinated annually.

Appendix VII – Wolf and dog diet in the study area at south of Douro river, expressed by identified prey items, number of prey detections, Frequency of Occurrence (F.O.), with correspondent classification of resource type, and Consumed Biomass.

Wolf		**N**	**F.O. (%)**	**Resource**	**Biomass (%)**
Wild Ungulates		9	3.8	-----	5.3
Sus scrofa	Juvenile	1	0.4	occasional	0.4
	Adult	8	3.3	supplementary	4.9
Domestic Ungulates		106	44.2	-----	58.5
Capra hircus		68	28.3	basic	27.3
Ovis aries		22	9.2	regular	8.3
Equus caballus		8	3.3	supplementary	10.3
Sus domestica		2	0.8	occasional	1.9
Bos taurus		6	2.5	supplementary	10.7
Lagomorphs		73	30.4	basic	20.7
Birds		37	15.4	-----	10.6
Galliformes		34	14.2	regular	9.7
Bird n.i.		3	1.3	supplementary	0.9
Carnivores		15	6.3	-----	4.0
Meles meles		6	2.5	supplementary	1.9
Canis familiaris		8	3.3	supplementary	2.8
Felis silvestris		1	0.4	occasional	0.3
Total		**240**	**100**		**100**

Dog		**N**	**F.O. (%)**	**Resource**	**Biomass (%)**
Wild Ungulates		10	4.2	-----	5.1
Sus scrofa	Juvenile	3	1.3	supplementary	1.1
	Adult	7	2.9	supplementary	4.0
Domestic Ungulates		168	70.0	-----	78.5
Capra hircus		95	39.6	basic	35.5
Ovis aries		56	23.3	basic	19.6
Equus caballus		7	2.9	supplementary	8.4
Sus domestica		2	0.8	occasional	1.8
Bos taurus		8	3.3	supplementary	13.3
Lagomorphs		20	8.3	regular	5.3
Small Mammals		1	0.4	occasional	0.3
Birds		30	12.5	-----	8.0
Galliformes		28	11.7	regular	7.4
Birds n.i.		2	0.8	occasional	0.5
Carnivores		10	4.2	-----	2.9
Meles meles		9	3.8	supplementary	2.6
Vulpes vulpes		1	0.4	occasional	0.3
Total		**239**	**100**		**100**

Appendix VIII - Regional variation (e.g. wolf pack) of wolf and dog diet in the study area at south of Douro river, expressed in Frequency of Occurrence (F.O) (N – number of prey detections in scats collected between 2014 and 2018).

Wolf		**Lapa**		**Leomil**		**Trancoso**	
Prey Class		**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**
Wild Ungulates		2	4.9%	4	3,3%	3	3,8%
Sus scrofa	Juvenile	0	0.0%	1	0.8%	0	0.0%
	Adult	2	4.9%	3	2.5%	3	3.8%
Domestic Ungulates		13	31.7%	35	29.2%	58	73.4%
Capra hircus		7	17.1%	23	19.2%	38	48.1%
Ovis aries		5	12.2%	3	2.5%	14	17.7%
Equus caballus		1	2.4%	6	5.0%	1	1.3%
Sus domestica		0	0.0%	1	0.8%	1	1.3%
Bos taurus		0	0.0%	2	1.7%	4	5.1%
Lagomorphs		6	14.6%	62	51.7%	5	6.3%
Birds		19	46.3%	13	10.8%	5	6.3%
Galliformes		18	43.9%	12	10.0%	4	5.1%
Birds n.i.		1	2.4%	1	0.8%	1	1.3%
Carnivores		1	2.4%	6	5.0%	8	10.1%
Meles meles		1	2.4%	2	1.7%	3	3.8%
Canis familiaris		0	0.0%	3	2.5%	5	6.3%
Felis catus		0	0.0%	1	0.8%	0	0.0%
Total		**41**	**100%**	**120**	**100%**	**79**	**100%**

Dog		**Lapa**		**Leomil**		**Trancoso**	
Prey Class		**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**
Wild Ungulates		4	9.3%	2	1.7%	4	5.2%
Sus scrofa	Juvenil	1	2.3%	1	0.8%	1	1.3%
	Adult	3	7.0%	1	0.8%	3	3.9%
Domestic Ungulates		20	46.5%	90	75.0%	58	76.3%
Capra hircus		14	32.6%	62	51.7%	19	25.0%
Ovis aries		4	9.3%	17	14.2%	35	46.1%
Equus caballus		0	0.0%	5	4.2%	2	2.6%
Sus domestica		0	0.0%	0	0.0%	2	2.6%
Bos taurus		2	4.7%	6	5.0%	0	0.0%
Lagomorphs		6	14.0%	8	6.7%	6	7.9%
Small Mammals		0	0.0%	0	0.0%	1	1.3%
Birds		13	30.2%	11	9.2%	6	7.9%
Galliformes		12	27.9%	10	8.3%	6	7.9%
Birds n.i.		1	2.3%	1	0.8%	0	0.0%
Carnivores		0	0.0%	9	7.5%	1	1.3%
Meles meles		0	0.0%	8	6.7%	1	1.3%
Vulpes vulpes		0	0.0%	1	0.8%	0	0.0%
Total		**43**	**100%**	**120**	**100%**	**76**	**100%**

Appendix IX - Chi-square test values to evaluate annual differences in the F.O. of each prey item in wolf and dog diet in the study area at south of Douro river.

	Wolf									
Prey class	**2014/2015**	**2015/2016**	**2016/2017**	**2017/2018**	**2014/2016**	**2014/2017**	**2014/2018**	**2015/2017**	**2015/2018**	**2016/2018**
Sus scrofa	12.80	8.06	15.18	15.18	15.18	🟥	15.18	12.80	8.06	8.87
Capra hircus	1.75	2.03	1.97	1.94	2.08	1.69	2.16	2.11	2.00	2.31
Ovis aries	5.32	2.87	2.87	5.80	2.97	6.23	10.11	4.55	5.03	2.93
Equus caballus	23.51	🟥	🟥	15.18	23.51	23.51	10.11	🟥	15.18	15.18
Sus domestica	31.84	🟥	🟥	🟥	31.84	31.84	31.84	🟥	🟥	🟥
Bos taurus	🟥	15.18	9.43	11.40	15.18	18.51	23.51	18.51	23.51	10.11
Lagomorphs	1.48	2.45	2.99	2.34	1.26	1.35	1.53	2.51	2.44	2.27
Galliformes	3.18	4.36	2.99	3.04	3.13	3.01	3.19	3.02	5.70	4.43
Birds n.i.	🟥	🟥	18.51	12.51	🟥	18.51	31.84	18.51	31.84	31.84
Carnivorous	6.50	6.26	15.18	11.02	9.43	18.51	7.74	8.53	5.86	7.38

	Dog									
Prey class	**2014/2015**	**2015/2016**	**2016/2017**	**2017/2018**	**2014/2016**	**2014/2017**	**2014/2018**	**2015/2017**	**2015/2018**	**2016/2018**
Sus scrofa	12.80	9.70	14.51	6.23	31.84	23.51	7.62	9.05	5.63	6.50
Capra hircus	1.64	2.03	0.77	0.99	1.35	1.41	1.59	1.04	2.24	2.18
Ovis aries	2.37	1.98	1.88	2.78	1.77	2.77	3.25	2.46	2.39	1.79
Equus caballus	12.80	7.68	7.68	12.80	12.80	12.80	🟥	7.68	12.80	12.80
Sus domestica	🟥	12.80	12.80	🟥	12.80	🟥	🟥	🟥	🟥	12.80
Bos taurus	24.50	5.09	5.68	23.51	5.46	98.50	19.30	31.84	14.51	4.95
Lagomorphs	3.67	20.10	14.51	13.02	3.60	3.55	3.55	16.50	16.50	14.51
Small Mammals	🟥	31.84	31.84	🟥	31.84	🟥	🟥	🟥	🟥	31.84
Galliformes	3.15	3.38	20.10	3.67	5.97	6.22	3.30	3.43	3.12	3.60
Birds n.i.	98.50	🟥	🟥	23.51	98.50	98.50	19.30	🟥	23.51	23.51
Carnivorous	13.93	20.10	14.51	6.23	12.50	11.40	6.00	16.50	6.81	6.50

🟨	Significant differences (p<0.05; 1g.l.)
🟥	**no occurrence in both years analysed**

Appendix X - Annual variation of wolf and dog diet in the study area at south of Douro river, expressed in Frequency of Occurrence (F.O) (N – number of prey detections in scats collected between 2014 and 2018).

Wolf		**2014**		**2015**		**2016**		**2017**		**2018**	
Prey Class		**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**
Wild Ungulates		0	0.0%	2	6.5%	2	5.6%	0	0.0%	5	6.3%
Sus scrofa	Juvenile	0	0.0%	1	3.2%	0	0.0%	0	0.0%	0	0,0%
	Adult	0	0.0%	1	3.2%	2	5.6%	0	0.0%	5	6.3%
Domestic Ungulates		23	30.7%	15	48.4%	21	58.3%	10	52.6%	37	46.8%
Capra hircus		15	20.0%	11	35.5%	11	30.6%	7	36.8%	24	30.4%
Ovis aries		3	4.0%	4	12.9%	8	22.2%	2	10.5%	5	6.3%
Equus caballus		3	4.0%	0	0.0%	0	0.0%	0	0.0%	5	6.3%
Sus domestica		2	2.7%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Bos taurus		0	0.0%	0	0.0%	2	5.6%	1	5.3%	3	3.8%
Lagomorphs		33	44.0%	8	25.8%	6	16.7%	4	21.1%	22	27.8%
Birds		15	20.0%	3	9.7%	5	13.9%	5	26.3%	9	11.4%
Galliformes		15	20.0%	3	9.7%	5	13.9%	4	21.1%	7	8.9%
Birds n.i.		0	0.0%	0	0.0%	0	0,0%	1	5.3%	2	2.5%
Carnivores		4	5.3%	3	9.7%	2	5.6%	0	0.0%	6	7.6%
Meles meles		2	2.7%	2	6.5%	0	0,0%	0	0.0%	2	2.5%
Canis familiaris		2	2.7%	1	3.2%	2	5.6%	0	0.0%	3	3.8%
Felis catus		0	0.0%	0	0.0%	0	0,0%	0	0.0%	1	1.3%
Total		**75**	**100%**	**31**	**100%**	**36**	**100%**	**19**	**100%**	**79**	**100%**

Dog		**2014**		**2015**		**2016**		**2017**		**2018**	
Prey Class		**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**
Wild Ungulates		0	0.0%	4	6.8%	1	3.4%	2	4.3%	3	11.1%
Sus scrofa	Juvenile	0	0.0%	3	5.1%	0	0.0%	0	0.0%	0	0.0%
	Adult	0	0.0%	1	1.7%	1	3.4%	2	4.3%	3	11.1%
Domestic Ungulates		49	62.8%	41	69.5%	24	82.8%	40	85.1%	14	51.9%
Capra hircus		34	43.6%	19	32.2%	6	20.7%	28	59.6%	8	29.6%
Ovis aries		14	17.9%	16	27.1%	10	34.5%	11	23.4%	5	18.5%
Equus caballus		0	0.0%	4	6.8%	2	6.9%	1	2.1%	0	0.0%
Sus domestica		0	0.0%	0	0.0%	2	6.9%	0	0.0%	0	0.0%
Bos taurus		1	1.3%	2	3.4%	4	13.8%	0	0.0%	1	3.7%
Lagomorphs		15	19.2%	1	1.7%	1	3.4%	2	4.3%	1	3.7%
Small Mammals		0	0.0%	0	0.0%	1	3.4%	0	0.0%	0	0.0%
Birds		10	12.8%	12	20.3%	1	3.4%	1	2.1%	6	22.2%
Galliformes		9	11.5%	12	20.3%	1	3.4%	1	2.1%	5	18.5%
Birds n.i.		1	1.3%	0	0.0%	0	0.0%	0	0.0%	1	3.7%
Carnivores		4	5.1%	0	0.0%	1	3.4%	2	4.3%	3	11.1%
Meles meles		4	5.1%	0	0.0%	0	0.0%	2	4.3%	3	11.1%
Vulpes vulpes		0	0.0%	0	0.0%	1	3.4%	0	0.0%	0	0.0%
Total		**78**	**100%**	**58**	**100%**	**29**	**100%**	**47**	**100%**	**27**	**100%**

Appendix XI - Chi-square test values to evaluate seasonal differences in the F.O. of each prey item in wolf and dog diet in the study area at south of Douro river.

	Wolf					
Prey class	**Winter/Spring**	**Winter/Summer**	**Winter/Autumn**	**Spring/Summer**	**Spring/Autumn**	**Summer/Autumn**
Sus scrofa	15.18	11.44	7.38	38.51	11.02	8.98
Capra hircus	1.99	2.62	1.57	2.06	1.87	1.65
Ovis aries	6.11	5.70	6.30	5.70	6.30	5.86
Equus caballus	10.11	14.51	23.51	10.95	15.18	31.84
Sus domestica	48.51	98.50	🟥	33.17	48.51	98.50
Bos taurus	12.80	98.50	31.84	11.51	9.70	24.50
Lagomorphs	1.52	1.61	1.61	2.36	2.36	2.43
Galliformes	7.68	2.63	4.07	2.63	4.07	2.65
Birds n.i.	48.51	31.84	🟥	20.10	48.51	31.84
Carnivores	6.52	7.83	6.30	9.70	7.06	8.68

	Dog					
Prey class	**Winter/Spring**	**Winter/Summer**	**Winter/Autumn**	**Spring/Summer**	**Spring/Autumn**	**Summer/Autumn**
Sus scrofa	10.53	12.51	10.53	12.51	10.53	12.51
Capra hircus	1.60	2.03	1.70	1.63	1.21	1.65
Ovis aries	2.31	2.37	2.42	2.12	2.18	3.00
Equus caballus	23.51	19.30	9.05	98.50	12.80	11.51
Sus domestica	31.84	98.50	🟥	24.50	31.84	98.50
Bos taurus	9.43	13.36	15.18	15.84	18.51	98.50
Lagomorphs	20.10	4.87	5.26	4.73	5.09	3.95
Small Mammals	🟥	98.50	🟥	98.50	🟥	98.50
Galliformes	5.68	4.30	3.43	7.62	4.11	3.49
Birds n.i.	🟥	98.50	48.51	98.50	48.51	33.17
Carnivores	20.10	10.50	10.50	9.70	9.70	7.68

🟨	Significant values (p=0.05; 1g.l.)

🟥	**no occurrence in both years analysed**

Appendix XII - Seasonal variation of wolf and dog diet in the study area at south of Douro river, expressed in Frequency of Occurrence (F.O) (N – number of prey detections in scats collected between 2014 and 2018).

Wolf		**Spring**		**Summer**		**Autumn**		**Winter**	
Prey Class		**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**
Wild Ungulates		0	0.0%	2	2.5%	3	7.9%	4	5.9%
Sus scrofa	Juvenil	0	0.0%	1	1.3%	0	0.0%	0	0.0%
	Adult	0	0.0%	1	1.3%	3	7.9%	4	5.9%
Domestic Ungulates		31	57.4%	32	40.0%	19	50.0%	24	35.3%
Capra hircus		18	33.3%	20	25.0%	15	39.5%	15	22.1%
Ovis aries		5	9.3%	8	10.0%	3	7.9%	6	8.8%
Equus caballus		3	5.6%	2	2.5%	0	0.0%	3	4.4%
Sus domestica		1	1.9%	1	1.3%	0	0.0%	0	0.0%
Bos taurus		4	7.4%	1	1.3%	1	2.6%	0	0.0%
Lagomorphs		14	25.9%	23	28.8%	7	18.4%	29	42.6%
Birds		5	9.3%	21	26.3%	6	15.8%	5	7.4%
Galliformes		4	7.4%	19	23.8%	6	15.8%	5	7.4%
Birds n.i.		1	1.9%	2	2.5%	0	0.0%	0	0.0%
Carnivores		4	7.4%	2	2.5%	3	7.9%	6	8.8%
Meles meles		2	3.7%	2	2.5%	0	0.0%	2	2.9%
Canis familiaris		1	1.9%	0	0.0%	3	7.9%	4	5.9%
Felis catus		1	1.9%	0	0.0%	0	0.0%	0	0.0%
Total		**54**	**100%**	**80**	**100%**	**38**	**100%**	**68**	**100%**

Dog		**Spring**		**Summer**		**Autumn**		**Winter**	
Prey Class		**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**	**N**	**F.O. (%)**
Wild Ungulates		2	5.4%	2	2.7%	2	4.7%	4	4.7%
Sus scrofa	Juvenile	0	0.0%	2	2.7%	0	0.0%	1	1.2%
	Adult	2	5.4%	0	0.0%	2	4.5%	3	3.5%
Domestic Ungulates		33	89.2%	46	62.2%	24	55.8%	65	76.5%
Capra hircus		19	51.4%	30	40.5%	12	27.9%	34	40.0%
Ovis aries		11	29.7%	13	17.6%	9	20.9%	23	27.1%
Equus caballus		0	0.0%	1	1.4%	3	7.0%	3	3.5%
Sus domestica		1	2.7%	1	1.4%	0	0.0%	0	0.0%
Bos taurus		2	5.4%	1	1.4%	0	0.0%	5	5.9%
Lagomorphs		1	2.7%	11	14.9%	6	14.0%	2	2.4%
Small Mammals		0	0.0%	1	1.4%	0	0.0%	0	0.0%
Birds		0	0.0%	9	12.2%	9	20.9%	12	14.1%
Galliformes		0	0.0%	8	10.8%	8	18.6%	12	14.1%
Birds n.i.		0	0.0%	1	1.4%	1	2.3%	0	0.0%
Carnivores		1	2.7%	5	6.8%	2	4.7%	2	2.4%
Meles meles		1	2.7%	4	5.4%	2	4.7%	2	2.4%
Vulpes vulpes		0	0.0%	1	1.4%	0	0.0%	0	0.0%
Total		37	100%	74	100%	43	100%	85	100%

Appendix XIII - Number of individuals reported to be killed by wolves (N Killed) and respective percentage (% K) according to declared wolf attacks to ICNF for each livestock species, in comparison with the number of prey detections in wolf and dog scats (N Diet) and respective proportion in F.O. values (% D), in the study area at south of Douro river during the same time period (2014-2017).

	N Killed	**% K**	**N wolf diet**	**% D (wolf)**	**N dog diet**	**% D (dog)**
Sheep	1176	78	17	23.6	51	33.5
Goats	191	13	44	61.1	87	57.2
Cattle	83	5	3	4.1	7	5.0
Equine	36	2	3	4.1	7	5.0
Asinine	22	1	0	0	0	0
Dogs	4	0.3	5	6.9	0	0
Total	**1512**	**100**	**72**	**100**	**152**	**100**